OPTIMIZING IMPACT ASSESSMENT OF ENTOMOLOGICAL INTERVENTION FOR MALARIA CONTROL IN AN OPERATIONAL SETTING IN ZAMBIA

by

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ABSTRACT

The study aimed at optimally assessing the impact of indoor residual spraying (IRS) and insecticide treated nets (ITNs) on vector species abundance, their infectivity and resistance status, and *Plasmodium falciparum* prevalence, malaria deaths and case fatality rates in the human population.

Malaria prevalence surveys were conducted and routine surveillance data was retrospectively analyzed. The average *P. falciparum* prevalence in children between the ages of 1 and 14 years was below 10% across the study period. The intervention effect was more pronounced in IRS areas than in ITNs localities but with an incremental protective effect of their combined use. Age-specific comparison showed better intervention effect on children below 5 years than older children 5 to 14 years old. While the average number of deaths and case fatality rates in children under the age of five plunged precipitately, the reductions were more significant in IRS districts than in ITNs districts. Results indicate the need for supplementing parasite prevalence survey data with routine surveillance data in low transmission intensity areas and demonstrate the significance of evidence-based age-specific deployment of interventions.

To monitor vector species abundance and infectivity, mosquitoes were collected daily using exit window traps. The three major vectors; *An. gambiae s.s, An. arabiensis* and *An. funestus s.s,* and three potential vectors of malaria, *An. nili, An. rivulorum* and *An. funestus*-like species were identified. Overall, the biggest impact of IRS and ITNs was on *An. gambiae* s.s, and *An. funestus* abundance. No *An. gambiae* s.s was collected in IRS localities, thus validating the fact that *An. gambiae s.s and An. funestus* are characteristically more amenable to control by IRS and ITNs than *An. arabiensis.* The transmission potential for all malaria vectors, as expressed by the calculated transmission index, was zero as none of the trapped mosquitoes tested positive for *P. falciparum* sporozoites. The identification of *An. nili, An. rivulorum* and *An. funestus*-like necessitate further research to determine their role in malaria transmission in the country. The low numbers of mosquitoes collected also indicate a compromise in the efficiency of exit window traps in low transmission settings, suggesting the need for their replacement with a more robust collection tool like the CDC light trap. While the persistence of *An. arabiensis* suggests the presence of resistance segregating in this population or, that this outdoor species is not in contact with IRS or ITNs, it could as well imply that it's the one species perpetuating malaria transmission in these meso-to hypo-endemic areas.

To determine the impact of interventions on insecticide resistance status of malaria vectors, susceptibility assays using the WHO standard protocol were conducted in 17 localities. High levels of resistance were detected in both *An, gambiae s.l* and *An, funestus s.l* to pyrethroids and DDT but with 100% susceptibility to malathion and bendiocarb. The level of resistance was significantly higher in IRS areas than in ITN areas. These findings indicate that resistance has been selected for following extensive vector control. Resistance to both DDT and deltamethrin in IRS localities and ITN areas with intense cotton growing was detected suggesting selection due to either historical use of DDT, gene flow or cross-resistance. All *An. gambiae s.s* were molecular s-forms and only the west (leu-phe) *kdr* was detected. Complete susceptibility to the organophosphates and carbamates provides a possibility to switch to these alternative insecticide classes for IRS. The detected increases in the malaria prevalence in localities with high insecticide resistance levels indicate vector control failure. These findings point to the need for information on underlying biochemical and molecular resistance mechanisms to make possible the design of an effective resistance management strategy, and for the assessment of the impact of resistance on interventions.

The results indicate that the impact of malaria control can be optimally assessed by using a combination of epidemiological (routine surveillance and prevalence data) and entomological indicators, in the context of a malaria decision support system, to enhance policy formulation for objective implementation of malaria control interventions and rational use of available resources.

DECLARATION

This work has not previously been accepted in substance for any degree and is not being currently submitted in candidature for any degree.

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This thesis is the result of my own investigations, except where otherwise stated. Other sources are acknowledged and a Bibliography is appended.

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TABLE OF CONTENTS

DEDICATION

To my beloved and cherished entire family, particularly for my father Dominic Bwalya Chanda in loving memory of my mother Elizabeth Chipasha Chanda.

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List of Figures

List of Tables

ACRONYMS

AChE	:	Acetylcholinesterase
ACT	:	Artemisinine-based Combination Therapy
AL	:	Artemether-Lumefantrine
ANVR	:	African Network on Vector Resistance
Bti	:	*Bacillus thuringensis* var. israelensis
CFR	:	Case Fatality Rate
CSO	:	Central Statistical Office
DALYs	:	Disability Adjusted Life Years
DDT	:	Dichloro-diphenyl-trichloroethane
DHS	:	Demographic Health Survey
DNA	:	Deoxyribonucleic Acid
ECZ	:	Environmental Council of Zambia
EIR	:	Entomological Inoculation Rate
ELISA	:	Enzyme-Linked Immunosorbent Assay
GABA	:	λ-aminobutyric acid
GDP	:	Gross Domestic Product
GPS	:	Global Positioning System
GST	:	glutathione-S- transferase
HCH	:	Hexachlorocyclohexane
HMIS	:	Health Information Management System
IGR	:	Insecticide Growth Regulator
IPT	:	Intermittent Presumptive Treatment
IRAC	:	Insecticide Resistance Action Committee
IRS	:	Indoor Residual Spraying
ITN	:	Insecticide Treated Net
ITS2	:	Internal Transcribed Spacer 2
IVCC	:	Innovative Vector Control Consortium
IVM	:	Integrated Vector Management
KAP	:	Knowledge Attitudes and Practices
Kdr	:	Knockdown resistance
LLIN	:	Longlasting Insecticidal Net
LSM	:	Larval Source Management

MDSS	:	Malaria Decision Support System
MFOs	:	Mixed Function Oxidases
MoH	:	Ministry of Health
NMCP	:	National Malaria Control Programme
NMSP	:	National Malaria Strategic Plan
PCR	:	Polymerase Chain Reaction
POPs	:	Persistent Organic Pollutants
RDTs	:	Rapid Diagnostic Tests
RBM	:	Roll Back Malaria
SNPs	:	Single Nucleotide Polymorphisms
SP	:	Sulphadoxine Pyrimethermine
SSP	:	Single Stranded Conformation Polymorphism
WHO	:	World Health Organization
WHOPES	:	World Health Organization Pesticides Evaluation Scheme

CHAPTER ONE

General Introduction and Literature Review

1.1 The Global Burden of Malaria

Vector borne diseases account for about 17% of the estimated global burden of infectious diseases (Townson et al. 2005) including Malaria parasite transmission which is of major public health significance worldwide. Approximately 3.2 billion people are at risk of Malaria disease with around 515 million cases (Breman et al. 2004) and 1 to 3 million deaths annually (Snow et al. 2005, Guerra et al. 2006). The first global campaign to combat malaria was the ill-fated World Health Organization's (WHO) - led malaria eradication programme that was conducted from 1956 to 1967 (Najera 1999, Utzinger et al. 2001). Although successful in some areas, in sub-Saharan Africa efforts proved unsustainable mainly due to malaria control associated technical and logistical challenges that were considered beyond the scope of the public health infrastructure in most African countries (Najera 1990). In 1992, the Global Strategy for Malaria Control was adopted at the Amsterdam Ministerial Conference, based on four strategic technical elements, including prevention and vector control, as a response to the increasing global malaria burden (Najera 1999). While malaria control efforts have been intensified in order to meet Roll Back Malaria, World Health Assembly and Millennium Development universal access and coverage targets, that aim to prevent, reduce or eliminate disease transmission (Komatsu et al. 2007), the disease still remains high on the international health agenda (Utzinger et al. 2001).

Malaria exacts its greatest toll in most sub-Saharan Africa countries were approximately 70% of the population resides in areas infested with potential malaria vectors (Hay et al. 2000). Africa has 60% of the morbidity and 90% of the mortality rates attributable to the disease (Breman et al. 2001, Breman et al. 2004). The disease affects mostly vulnerable individuals i.e. children under the age of five and pregnant women (Baird et al. 1998, Kleinschmidt and Sharp 2001). About 1 million children below 5 years of age from this region die each year from malaria related illness, constituting nearly 25% of overall child mortality (Snow et al. 1999, Breman and O'Meara 2005). This colossal burden of the disease has translated into economic loses for both individuals and health systems. WHO estimates indicate that close to 45 million disability-adjusted life years (DALYS) are lost due to malaria in Africa (WHO 2001). The economic burden of malaria culminating in slower economic

development is reflected in work- or school- days lost due to malaria and the consequences of malaria in pregnancy and in children less than five years of age. The regional estimates suggest a deficit of 1.5% in Gross Domestic Product (GDP) in the affected Countries. In Africa alone, malaria would account for 12 billion US Dollars due to health care related costs and a reduction in the production potential due to an episode of malaria (Breman and O'Meara 2005, WHO 2005). Thus, the disease continues to be a major impediment to social-economic development in resource constrained countries, especially in sub-Saharan Africa (Sachs and Malaney 2002).

1.1.1 The Transmission and Distribution of Malaria

The global transmission range of malaria is uneven, but concentrated in more than 100 countries within the tropical and subtropical regions of the world (Sachs and Malaney 2002). Here the disease remains a major contributor to high childhood morbidity, mortality, malnutrition, anaemia and stunted growth (McClean and Senthilselvan 2002). However, indigenous malaria has been recorded as far north as 64^{o}N latitude (Archangel in the USSR) and as far south as 32^{o}S latitude (Cordoba in Argentina) following the 16 degrees Celsius summer isotherm limit. In terms of vertical distribution, the disease has occurred in the Dead Sea area at 400m below sea level, and at Londiani (Kenya) at 2591m above sea level. Within these latitude and altitude limits, there are large areas that are free of malaria (Bruce-Chwatt 1985). Although the geographical distribution of malaria transmission is confined to the tropics and sub-tropical areas, it formerly extended to the temperate regions such as northern Europe and North America (Sachs and Malaney 2002).

While the disease is essentially focal in nature with transmission depending greatly on local environmental and other conditions (Bruce-Chwatt 1985), in most sub-Saharan Africa countries the problem is exacerbated by several compounding factors. These include physiological factors, such as presence of anti-malaria drug-resistant parasites (Sweeney 1996, White 1998, Barnes et al. 2008) and insecticide-resistant vectors (Collins and Paskewitz 1995, Hemingway and Ranson 2000, Roberts and Andre 1994). Socio-ecological factors include environmental changes as a result of irrigation agriculture and construction (Keiser et al. 2005), increased

population and human migration (Martens and Hall 2000), slower economic development (Sachs and Malaney 2002), political upheaval, poverty levels and dilapidated health services (Greenwood and Mutabingwa 2002). Environmental conditions, climatic changes - global warming, floods associated with rains and natural disasters also contribute to changes in disease transmission (Lindblade et al. 1999). Other factors that may be relevant are the adaptability of malaria vectors (*An. gambiae* complex) to changing environments (Chinery 1984, Mc Wilsons et al. 1999, Chinery 1984, Mc Wilsons et al. 1999) and limited investment in research, drug discovery and optimisation of malaria vector control programmes.

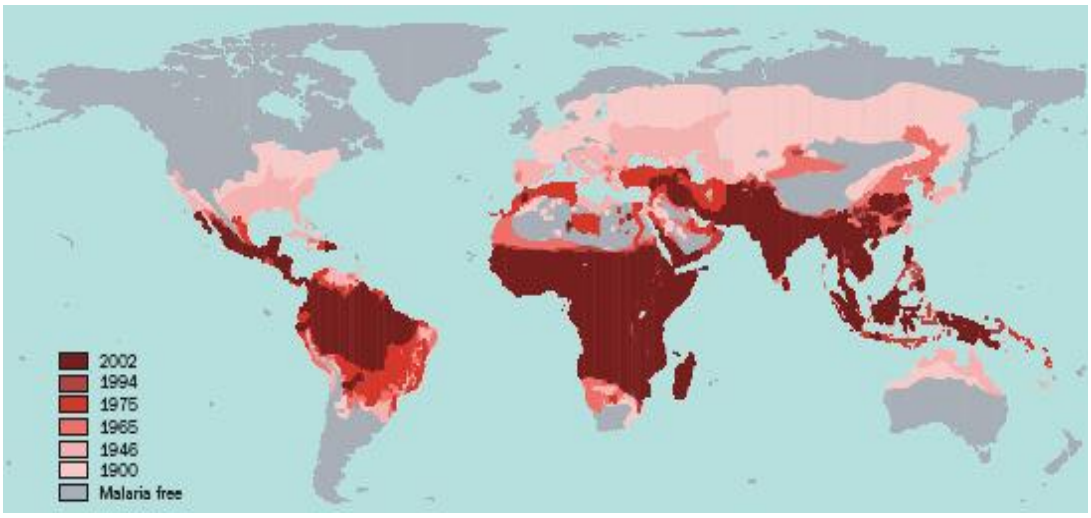


Figure 1.1: The global distribution of malaria since pre-intervention (1900-2002). (Picture adapted from (Hay et al. 2004)).

1.1.2 Historical Perspective of Malaria Control

Malaria is a preventable disease and with timely definitive diagnosis and adequate effective treatment the disease is curable. The inaugural malaria control strategies followed in the wake of the elucidation of the life cycle of the malaria parasite in the Anopheline mosquito and in the human as a result of the discovery of the protozoan parasite by Lavaran in 1880 and its transmission by the mosquito vector by Ross in 1897 and Grassi et al. in 1898 (Bruce-Chwatt 1985). Historical treatment of the fevers associated to malaria date back 2000 years in China, and included the use of an infusion of ginghaosu (*Artemisia annua*) and the bark of the *cinchona ledgeriana* tree, in Peru by 1600, from which quinine was derived and later developed into the safer and cheaper chloroquine. The first vector control interventions against malaria were carried out by the Romans by means of drainage programmes, after observing

the association of disease with standing water (Bruce-Chwatt 1985).

The elucidation of the malarial life cycle facilitated the definition of the key attributes of the epidemiology of malaria and enabled the implementation of targeted control interventions (Gilles and Warrell 1993). With the discovery of dichloro-dimethyl-trichloroethane (DDT) in 1942 and its successful utilization for malaria control in 1944 in Italy, the prospects of global eradication of malaria seemed feasible (Najera 2000). In 1955, the Global Malaria Eradication campaign was launched by the 8th World Health Assembly for all malaria endemic countries with the exception of Madagascar and sub-Saharan Africa (Najera 1999). The campaign was built on the twin pillars of case management with chloroquine, sulphadoxine/ pyrimethamine and quinine, and vector control using DDT intra-domiciliary spraying (WHO 1957). The WHO-backed global programme eliminated the disease in 37 out of 143 endemic countries by 1978, of which 27 were in Europe or the Americas (WHO 2008, Bruce-Chwatt 1985). Elsewhere this "time-limited" eradication program proved unsuccessful. The development of drug and insecticide resistance coupled with financial constraints and lack of public health infrastructure for implementation in the tropics derailed the efforts in sub-Saharan Africa (Najera 1999). This resulted in a resurgence in malaria morbidity and mortality from the early 1970s, and by 1976 the strategy had shifted into long-term integrated control through primary health care programs (WHO 2008).

Following the striking increase in disease burden, there was an obligation to re-establish malaria control as a global health priority (WHO 2003). The Roll Back Malaria partnership was created in 1998, to coordinate global efforts in combating malaria (WHO 1993). This was linked to resurgence in anti-malaria activities and an unparalleled increase in funding. In response, the malaria burden dropped and the global population at risk of malaria decreased from 77 % in 1900 to 48 % in 2002 (Hay et al. 2004)(Figure 1.1). While several drugs are available for effective treatment of malaria (Ridley 2002), only 60% of all malaria patients have prompt access to appropriate treatment within 24 hours of the onset of symptoms (WHO 2005). Prevention through intermittent presumptive treatment (IPT) for pregnant women and vector control for all, therefore remains a priority for most malaria control programmes.

1.2 Classification and Distribution of Malaria Vectors

Mosquitoes belong to the family Culicidae in the order Diptera, class Insecta, Phylum Arthropoda (Darsie and Ward 2005, Darsie and Ward 2005). Culicidae is divided into three subfamilies Anophelinae, Culicinae, and Toxorhynchitinae, and comprises approximately 3450 recognized species of mosquitoes in 38 genera. The 34 genera are in the subfamily Culicinae, 3 in Anophelinae and only 1 in Toxorhynchitinae (Foster and Walker 2002). Although climate is the major factor governing distribution and relative abundance of insects (Andrewartha and Birch 1954), other factors such as local climate effects, salinity of breeding sites and the relative availability of different host species are also important (Sutherst and Maywald 1995). The distribution of major vectors of malaria is determined mainly by temperature and the capacity of the air to desiccate the insect (Lindsay et al. 1998).

Malaria vectors belong to the genera *Anopheles* (Cellia) Myzomyia and their global distribution has been recognized in six zoo-geographical regions; Palaearctic, Oriental, Australasian, Afro-tropical, Neoarctic and Neotropical regions (Hackett 1937, Bruce-Chwatt 1985). Approximately 460 species of *Anopheles* mosquitoes have been identified throughout the world, many of which are species complexes. Only about 80 species are capable of transmitting malaria, 70 species are vectors of malaria under natural conditions and approximately 45 are of major significance (Foster and Walker 2002).

The global distribution of principal vectors of malaria (Figure 1.2) is associated with 12 epidemiological zones of malaria: North America, Central America, South America, Afro-tropical, North Eurasian, Mediterranean, Afro-Arabian, Indo-Iranian, Indo-Chinese Hills, Malaysian, Chinese and Australasian (Macdonald 1957, Bruce-Chwatt 1985). Africa can be divided into six eco-epidemiological strata, plus a "special" category:

- *West and Central Sahel*: Mauritania, Senegal, Mali, Niger, Chad, northern Sudan (Short season)
- *Horn countries*: Djibouti, Eritrea, Ethiopia, and Somalia (Short season)

- *Southern Africa*: Botswana, Comoros, Madagascar, Namibia, South Africa, Swaziland, Zambia, Zimbabwe, southern Angola and Mozambique
- *Highland area:* Land at or above 1000 meters Above Sea Level with limited seasonal transmission:
- Epidemic-prone Cities, towns and large villages, in-hospitable to *Anopheles.*
- *Rest of tropical Africa*: Transmission during most months of the year in forest and *savanna.*
- *Special transmission paradigms*: In irrigated areas, plantations, industrial mines and other "organized" communities (Macdonald M. B, unpublished data).

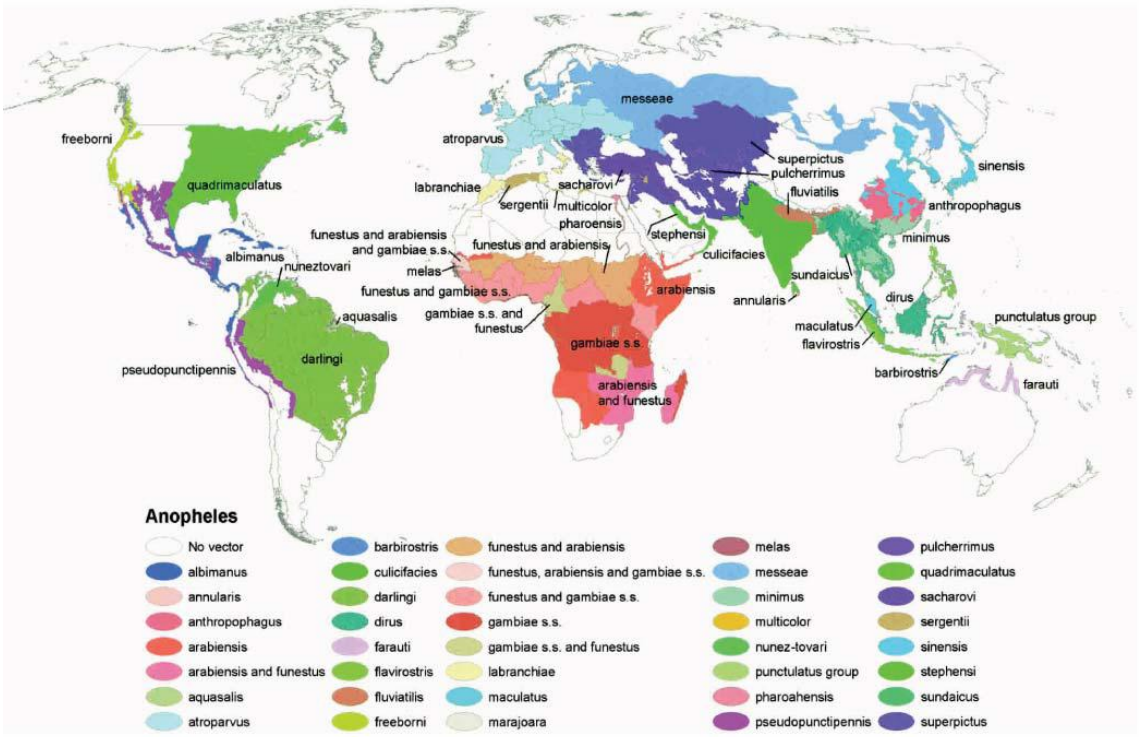


Figure 1.2: Global distribution of dominant malaria vectors. (Picture adapted from (Kiszewski et al., 2004))

1.2.1 Afro-tropical Anopheline Complexes

While, several *Anopheles* species have been incriminated in malaria transmission in sub-Saharan Africa, the exceptionally high transmission rates in the region are in large part ascribed to the constant presence of efficient and competent mosquito vectors with high vectorial capacity. Most of these species belong to the biologically

diverse taxa of mosquitoes: *Anopheles* (Cellia) *gambiae* Giles complex and *Anopheles (Cellia) funestus* Giles group (Zahar 1985). Each of the two complexes is a group of morphologically indistinguishable yet genetically and behaviourally distinct sibling species that vary dramatically in their efficiency to transmit malaria (Hackett 1937, White 1974, Coluzzi et al. 1979, Hunt et al. 1998). Historical studies have shown that the most prevalent and key malaria vectors in sub-Saharan Africa are *Anopheles gambiae* Giles 1902 and *Anopheles arabiensis* Patton in the *An. gambiae* complex, and *Anopheles funestus s.s,* Giles, 1900 in the *An. funestus* complex (Gillies and De Meillon 1968, White 1974, Mouatcho et al. 2007) with *An. merus, An. bwambae* and *An. nili* implicated in transmission in localized areas (Krafsur 1970, Gillies and Coetzee 1987, Besansky et al. 2004).

1.2.1.1 The *Anopheles gambiae* Complex

The *An. gambiae* complex consists of at least seven morphologically indistinguishable and largely sympatric (geographically co-existing) species (White 1974, Coluzzi et al. 1979, Coluzzi et al. 2002, Ayala and Coluzzi 2005, Hunt et al. 1998, Wang-Sattler et al. 2007). The *An. gambiae* complex in Africa comprises three species that breed in fresh-water (*An. arabiensis* Patton, *An. gambiae s.s., An. quadriannulatus A* Theobald from South Africa (White and Rosen 1973) and two salt-water breeders *An. melas* Theobald in West Africa and *An. merus* Dönitz in East Africa (Berzosa et al. 2002). Both species are mostly zoophilic but also bite humans in the absence of animals (Coetzee et al. 2000). A sixth member, the halophilic *An. bwambae* White that breeds only in mineral water has been described in Uganda, where it co-exists with *An. gambiae s.s.* as adult mosquitoes, and is an important local vector (White and Rosen 1973, Scott et al. 1993). The *An. gambiae* complex now includes seven species with the recent description of another fresh water species *An. quadriannulatus* B from Ethiopia (Hunt et al. 1998). While *An. gambiae s.s.,* 1902 and *An. arabiensis* are major malaria vectors, widely distributed in sub-Saharan Africa, *A. quadriannulatus* species A and B are not vectors, due to their strong zoophily and exophily (Gillies and De Meillon 1968). The complex is collectively known as *Anopheles gambiae s.l* (Sensu lato).

1.2.1.2 Molecular and Chromosomal forms

An. gambiae, s.s is the most efficient Afro-tropical malaria vector; it is anthropophilic, in that it possesses a remarkable tendency to feed preferentially on humans. It is a long-lived species and breeds through out the year. The temperatures in the tropics are ideal for parasite proliferation and hence, *An. gambiae* contributes significantly to the stability of malaria transmission in sub-Saharan Africa. In West Africa, *An. gambiae* has been divided into five chromosomal forms designated with a non-Linean nomenclature: Bamako, Mopti, Savanna, Forest and Bissau (Coluzzi et al. 1985, Toure et al. 1994, Toure et al. 1998, Wondji et al. 2002). Their geographical distribution and ecological data is associated with particular climatic zones with Mopti, Savannah and Bamako more adapted to dryer environments, but they breed all year long in irrigated fields. These three forms are often sympatric and their distributions overlap with that of *An. arabiensis*, while Forest and Bissau forms are exclusively found in more humid forested areas. To provide more insights into their taxonomic status, recent efforts have focused on the pattern of variation observed with molecular markers. This revealed the existence of two genetic variants referred to as the molecular M and S forms (Favia et al. 1994, Della Torre et al. 2001, Della Torre et al. 2002, Wondji et al. 2002). Both forms are anthropophagic and effective vectors of human malaria parasites (Awolola et al. 2005).

1.2.1.3 The *Anopheles funestus* Complex

The *Anopheles (Cellia) funestus* group is composed of nine members and is divided into two African subgroups: (Funestus subgroup includes *Anopheles aruni* Sobti, *Anopheles confusus* Evans and Leeson, *Anopheles funestus s.s*, *Anopheles parensis* Gillies, *Anopheles vaneedeni* Gillies and Coetzee; Rivulorum subgroup includes *Anopheles brucei* Service, *Anopheles fuscivenosus* Leeson, *Anopheles leesoni* Evans, *Anopheles rivulorum* Leeson, and an "*An. rivulorum*-like" species) (Koekemoer et al. 2002, Cohuet et al. 2003, Mouatcho et al. 2007, Spillings et al. 2009). These species exhibit exceptional morphological overlap, and are distinguishable based on attributes of specific developmental stages vis as vis eggs and larvae (Gillies and De Meillon 1968, Gillies and Coetzee 1987). Their biology and vectorial capacity is highly contrasting (Cohuet et al. 2003). Nevertheless *An. funestus s.s*, is an essentially anthropophilic and endophilic species and is the only

member of the complex recognized as a significant malaria vector in Africa (Gillies and De Meillon 1968).

An. rivulorum is primarily zoophilic, but was found infected with *P. falciparum* and is only a minor vector at a localized site in Tanzania (Wilkes et al. 1996, Koekemoer et al. 2002, Cohuet et al. 2003). *An. vaneedeni* has been experimentally infected in the laboratory with *P. falciparum* but its vectorial role has never been demonstrated in nature, and it is thus not implicated in malaria transmission (De Meillon et al. 1977, De Meillon et al. 1977). The other members of the *An. funestus* group are mainly zoophilic and do not seem to be involved in the transmission of malaria. The distribution of *An. funestus* is widespread throughout subtropical Africa, and can be found in sympatry with other members of the complex (Gillies and Coetzee 1987). A new species in this group provisionally named as *An. funestus*-like has recently been described in Malawi (Spillings et al. 2009) although its involvement in malaria transmission is yet to be established.

1.2.1.4 The *Anopheles nili* Complex

The *An. (Cellia) nili* complex comprises four member species: typical *An. nili* (Theobald, 1904), *An. carnevalei* Brunhes et al, *An. somalicus* Rivola and Holstein and *An. ovengensis* (Awono-Ambene et al. 2004, Fontenille and Simard 2004, Kengne et al. 2003). The members of this group exhibit extensive morphological, ecological and ethological variations (Gillies and De Meillon 1968, Carnevale et al. 1992, Brunhes et al. 1999). They can be distinguished through slight morphologic diagnostic characters, observable at the larval and/or adult stages (Awono-Ambene et al. 2004, Brunhes et al. 1999), and a molecular diagnostic tool based on segregating sequence differences in the Internal Transcribed Spacer 2 (ITS2) of the ribosomal DNA (rDNA)(Kengne et al. 2003). Of the four species, *An. nili s.s.* is the most important malaria vector although *An. carnevalei* and *An. ovengensis* have been found infected with *P. falciparum* in natural conditions (Antonio-Nkondjio et al. 2006, Awono-Ambene et al. 2004, Awono-Ambene et al. 2009). *Anopheles somalicus* is mainly zoophilic and highly exophilic, it is not involved in human malaria transmission (Gillies and De Meillon 1968, Rivola and Holstein 1957).

Anopheles nili s.s is a widespread efficient vector of malaria in humid and forested areas across tropical Africa (Antonio-Nkondjio et al. 2006, Ayala et al. 2009, Carnevale et al. 1992, Dia et al. 2003, Moffett et al. 2007) and has been described as a major vector in West and Central Africa (Hamon and Mouchet 1961) and secondary vector of malaria to *An. gambiae* and *An. funestus* in East Africa (Krafsur 1970). This species is highly anthropophilic, endophagic and exophilic. Larvae of *An. nili* are typically found in vegetation or debris or in dark shade along the edges of sun-lit fast running streams and large rivers (Awono-Ambene et al. 2004, Antonio-Nkondjio et al. 2006, Carnevale et al. 1992, Dia et al. 2003). The review of the bionomics and vectorial status of *An. nili*; shows that virtually all information available concerns West or Central African forms and *An. nili* from other regions remain poorly known (Gillies and De Meillon 1968, Ndo et al. 2010).

1.3 Malaria vector bionomics, ecological variations and implications for their control

Malaria epidemiology is influenced not only by favourable climatic factors for mosquito breeding and parasite development, but also by the availability of both efficient vectors and the human host, whose density is crucial in determining the malaria risk (Garret-Jones 1964, Moffett et al. 2007). Malaria transmission by competent vectors mainly depends on frequency of sporozoites in the mosquito, ability to feed on human hosts (anthropophagic) (Kiszewski et al. 2004) and longevity to survive sporogony, i.e. the time required for sporozoite development in the mosquito (Macdonald 1957, Gillies 1988).

Mosquito longevity is a pivotal factor in vector control, as demonstrated through mathematical models (Ross 1911, Macdonald 1957, Killeen et al. 2000, Killeen et al. 2001, Smith and McKenzie 2004, Bayoh and Lindsay 2004, Le Menach et al. 2005, Le Menach et al. 2007), and is dependent on blood and sugar feeding behaviour, environmental factors, including availability of hosts and breeding sites (Killeen et al. 2004, Minakawa et al. 2006, Killeen and Smith 2007, Manda et al. 2007), humidity (60% to 80%) and temperature (Lindblade et al. 2000). While temperatures between 22°C and 32°C with monthly rainfall of about 80mm for at least five months per year are ideal climatic conditions for stable malaria transmission, those below 18°C are considered unsuitable for transmission (Craig et

al. 1999). However, *An. arabiensis* is able to survive at higher temperatures than *An. gambiae* s.s. (Kirby and Lindsay 2004).

Additional attributes of the principal malaria vectors relate to their biting time, if they bite in the night or at dawn or dusk (nocturnal or crepuscular), if they bite indoors or outdoors (endophagic or exophagic), if they tend to rest indoors or outdoors (endophilic or exophilic), if they prefer animal or human hosts (zoophagic or anthropophagic), their flight range as well as their preferred larval habitats (Gillies et al. 1968, White 1974, Gillies and Coetzee 1987, Pates and Curtis 2005).

1.3.1 Larval ecology of malaria vectors

Mosquitoes have three aquatic stages: egg, four different instars of larvae and pupae. Progress from egg to adult takes about six days under optimal climatic conditions (Gillies and De Meillon 1968). Larval survival is dependant on a number of factors, including temperature, water quality, nutrient competition, predation and disease including dispersal (Depinay et al. 2004). Temperature has been found to be the most critical variable in malaria epidemiology (Depinay et al. 2004, Fillinger et al. 2004). However, the temperature for larval survival does not equate to the optimal temperature for rapid development, with the former being lower than the latter (Bayoh and Lindsay 2003, Bayoh and Lindsay 2004). This occurs because there is a linear relationship between water temperature and larvae maturation time, while larval survival rates are non linear and reach saturation at high temperatures (Hoshen and Morse 2004). Shelton (1973) reported how larvae that survive high temperatures produce smaller, less successful adults. At high temperatures a large proportion of larvae died at pupation, or pupae failed to emerge into adults, a finding attributed to disruption of the highly complex process of metamorphosis (Clements 1992, Chambers and Klowden 1990). There seems to be systematic cell death at high temperature. As the body temperature of an insect rises, the rates of both metabolism and respiration increase up to a critical thermal limit, and death occurs soon after respiration begins to drop, even if the insect is returned to normal temperatures (Neven 2000).

An understanding of the ecology of the vector informs the design of effective

malaria control strategies. Larval abundance and distribution are important factors affecting successful control of adults and larvae. Muirhead-Thomson (1951) found that *An. gambiae s.l* larvae develop in fresh water habitats that are small, temperary, clean and exposed to sunlight. These are not the only type of habitats encountered by these vectors. Holstein (1954) argued that it is difficult to attribute a definite type of breeding place to *An. gambiae s.l* and that this vector species complex can potentially breed in almost any fresh or brackish water body that happens to be available. The adaptation to more urban situations by *An. gambiae s.l* was observed by Chinery (1984). Fillinger et al. (2004) point out that the flexibility of this species complex should never be underestimated. In operational larval control programmes high effective coverage is necessitated by high levels of endemicity. Preferences for breeding sites are governed by a diverse set of physical parameters including: Water movement, temperature, amount of light and shade, chemical factors, like dissolved oxygen, nitrates, alkalinity, pH and dissolved solids (Haddow 1943). While many factors may have an effect on the quality of the breeding site, only a few are important for a specific species (Muirhead-Thomson 1951). For example, a strong correlation was observed between the presence of different *Anopheles* species and the presence of different vegetation types (Bogh et al. 2003, Fillinger et al. 2004, Minakawa et al. 2004).

Oviposition and larval breeding site preferences often vary substantially between mosquito species, even when they are closely related. For example, the M and S form of *An. gambiae* s.s occupy distinct niches (Della Torre et al. 2001, Wondji et al. 2002). *An. gambiae s.l.* mainly prefers shallow, open, sunlit habitats like rice fields, borrow pits and stagnant water such as pools, puddles and hoof prints (Gillies et al. 1968, Gillies and Coetzee 1987, Service 2000). They often utilize small temporary pools due to higher water temperature and lower predation (Service 1971, Minakawa et al. 2001, Minakawa et al. 1999, Minakawa et al. 2004, Gimnig et al. 2001). *An. funestus,* in comparison, prefers shade and is therefore found in more permanent water bodies with vegetation such as marshes, river edges or rice fields with mature plants providing shade. *An. merus* and *An. melas* in contrast breed in brackish lagoons, ponds, swamps, pools and puddles with 50% to 75% seawater. *An. quadrianulatus A and An. quadrianulatus B,* and *An. funestus* generally prefer clean and unpolluted water bodies (Gillies et al. 1968, Service 2000). Alarmingly,

adaptation of *An. gambiae* to breed in brackish water has been reported in West Africa (Bogh et al. 2003, Chinery 1984). These differences in adaptation for fresh- and brackish-water cause spatial segregation between adult members of the *An. gambiae* complex (Bryan et al. 1982).

1.3.2 Adult ecology of malaria vectors

The range and relative abundance of major malaria vectors is strongly influenced by climatological factors, particularly annual precipitation (Lindsay and Martens 1998). Spatial and temporal fluctuations in their densities are seasonal and coincide with rainfall patterns (Rogers et al. 2002, Cohuet et al. 2004). For example, *An. gambiae* and *An. funestus* are more dominant in wet and humid areas, whilst *An. arabiensis* is better adapted to drier conditions and predominate in arid savannas (White 1974, Lindsay and Martens 1998, Coetzee et al. 2000). In areas where *An. arabiensis* and *An. gambiae* co-exist, there are huge heterogeneities in densities, with the former predominating during the dry season and the later becoming more abundant in the rainy season (Di Deco et al. 1981, Gillies et al. 1968, Smith et al. 1993, Takken et al. 1998, Kulkarni et al. 2006) or vice-versa (Service 1971, White and Rosen 1973). The density of adult *An. funestus* populations vary in relationship with rainfall (Rogers et al. 2002). Its densities begin to increase in the middle of the rainy season and become more abundant at the commencement of the subsequent dry season (Gillies et al. 1968, Smith et al. 1993). However, where annual precipitation is throughout the year, and streams are permanent, the species is always present (Lindsay and Martens 1998). While directly influenced by abundance of larval habitats and ideal temperature and humidity, malaria transmission is strongly dependent on the density of older sporozoite infected mosquitoes, rather than overall vector population size (Gillies et al. 1968, Gillies et al. 1968). This results from the huge numbers of non infectious young mosquitoes during the peaks of mosquito abundance. However, both the mean age and the proportion of sporozoite infected mosquitoes increases with the decline of densities (Charlwood et al. 1995, Shiff et al. 1995, Shililu et al. 2004, Kulkarni et al. 2006).

Several studies have been conducted on malaria prevalence and mosquito abundance relative to their proximity to larval habitats (Lindsay et al. 1991, Boudin et al. 1992, Faye et al. 1993, Lindsay et al. 1993, Lindsay et al. 1995, Smith et al. 1995, Lindsay

et al. 2000). In areas where the major larval habitat was a river, large swamp or rice field, decreasing mosquito abundance was observed with distance from the breeding sites (Lindsay et al. 1995, Ribeiro et al. 1996, Thomas and Lindsay 2000, Minakawa et al. 2002, Diuk-Wasser et al. 2005, Cano et al. 2006, Bogh et al. 2007). Other studies have demonstrated lower malaria prevalence in areas closer to rice fields and rivers than in those situated further away (Lindsay et al. 1991, Boudin et al. 1992, Thomas and Lindsay 2000, Ijumba and Lindsay 2001, Diuk-Wasser et al. 2005). This paradox is supported by models postulating that the phenomena is an effect of distantly located water accumulations, that act as an oviposition site from which infected mosquitoes reinitiate the search for blood (Le Menach et al. 2005), thus leading to increased proportions of infectious mosquitoes with distance from their location of actual emergence (Smith and McKenzie 2004).

The principle malaria vectors are quite discriminating in their biting and resting behaviours, which has implications for vector control. *An. gambiae s.l.* and *An. funestus* are highly endophagic and endophilic (Gillies and DeMeillon 1968, Gillies and Coetzee 1987) nocturnal feeders with maximum biting taking place between midnight and 4:00 am, but continuing until just after sunrise (Haddow 1943, Gillies and DeMeillon 1968, Surtees 1970, Lindsay et al. 1989, Dossou-Yovo et al. 1999). *An. arabiensis* behaviour is more varied than that of *An. gambiae.* It can feed and rest both indoors and outdoors due to its zoophilic behaviour (Shililu et al. 2004, Kulkarni et al. 2006). *An. arabiensis* feeds more readily on cattle than *An. gambiae s.s.* While the period for blood-feeding is genetically-fixed, extensive vector control through insecticide-impregnated bednets and indoor residual spraying reduces vector survival and suppresss vector populations (Magesa et al. 1991, Gimnig et al. 2003, Sampath et al. 1998) and may alter foraging behaviour (Fornadel et al. 2010).

The extensive use of IRS and ITNs may select for vectors that feed at other times (Rishikesh 1966). ITNs can also shift anopheline biting outdoors (Magesa et al. 1991, Mbogo et al. 1996), earlier in the evening (Magesa et al. 1991, Mbogo et al. 1996, Charlwood and Graves 1987) or onto alternate hosts (Sampath et al. 1998, Charlwood and Graves 1987, Bogh et al. 1998). In Ethiopia peak biting by *An. arabiensis* was early in the night (20:00 to 22:00 hours), mainly before people went to bed (Abose et al. 1998, Yohannes et al. 2005). Some insecticides may also have

contact irritancy and/or non-contact excito-repellancy effects, decreasing the numbers of *An. gambiae s.l* that enter sleeping quarters and causing mosquitoes that do enter to exit more quickly (Lines et al. 1987, Miller et al. 1991). Following eight years of insecticide spraying in Zimbabwe, a shift from endophagy to exophagy was observed in *An. gambiae s.l.* (Muirhead-Thomson 1960). Equally, the impact of ITNs in reducing indoor biting has been widely demonstrated (Karch et al. 1993, Mbogo et al. 1996, Cuzin-Ouattara et al. 1999, Ilboudo-Sanogo et al. 2001, Takken 2002). House design and personal protection matters may also vary within a village, causing strong variations in biting rates between households (Lindsay et al. 2002).

1.4 Contemporary Malaria Vector Control Interventions

Malaria remains a leading cause of morbidity and mortality in sub-Saharan Africa (Snow et al. 2005). Recently global efforts to combat the disease have been increased (WHO 2009). The huge malaria disease burden can in large part be attributed to inadequate preventive measures for the vulnerable, particularly children under the age of five years and pregnant women (Gamble et al. 2006, Brooker et al. 2006). Consensus on policy and strategy has stimulated unprecedented political-will in malaria endemic countries, backed by international organizations and donors, culminating in setting of increasingly ambitious targets for control: that is, to achieve at least 80% coverage of key interventions by 2010 and reduce morbidity and mortality by 50% by 2010 and 75% by 2015 respectively (Komatsu et al. 2007, WHO 2008). The launch of Roll Back Malaria (RBM) in 1998, the United Nations Millennium Declaration in 2000, the Abuja Declaration by African Heads of State in 2000, the World Health Assembly in 2005, and the RBM global strategic plan 2005–2015 have all contributed to the establishment of goals, indicators and targets for malaria control (WHO 2008, WHO 1993). This includes the ways of measuring progress towards these goals by member countries (WHO 2007, RBM 2000, WHO 2008).

Vector control has a proven record of contributing to the reduction of vector-borne disease transmission (WHO 2004, Townson et al. 2005). There is no effective vaccine for most important vector borne diseases, including malaria. The only way

to control these diseases in highly endemic areas is to prevent transmission by insect vectors. Vector control, personal protection and community participation are the pillars of the WHO strategies for insect-transmitted disease control. Unfortunately, mass malaria chemo-prophylaxis cannot be implemented for technical and economic reasons, especially in Africa. The effective treatment of malaria cases is increasingly complex and expensive because of drug resistance. In high-transmission areas (which include most parts of Africa) malaria incidence cannot be reduced if, in parallel with early diagnosis and treatment, transmission is not controlled through very effective vector-control and/or personal-protection interventions.

Most endemic countries have implemented a double pronged approach to malaria control, with effective case management using artemisinin based combination therapy (ACT) and reducing vector-human contact with vector control (Kleinschmidt et al. 2007, Sharp et al. 2007, Protopopoff et al. 2007). The main objective of malaria vector control is to significantly reduce the incidence and prevalence of both parasite infection and clinical malaria by controlling the malaria-bearing mosquito and thereby reducing and/or interrupting transmission (WHO 2008). There are two refined mainstream interventions for contemporary malaria vector control: The use of indoor residual spraying (IRS) and insecticide treated bed nets (ITNs) (Pluess et al. 2010, Yukich et al. 2008, Lindsay et al. 1989, Roberts et al. 2004, Kleinschmidt et al. 2009, WHO 2006, WHO 2007). The efficacy of these two methods as malaria vector control tools have been evaluated in different epidemiological settings (Lengeler and Sharp 2003) at experimental field trial (Lengeler 2004, Mabaso et al. 2004) and community-wide (Curtis and Mnzava 2000, Lines et al. 2003) levels. These interventions may be complimented in specific locations, by other methods such as larviciding or environmental management (WHO 2008).

Malaria transmission in sub-Saharan Africa is mainly perpetuated by the constant presence of the three major vectors of the disease, *An. gambiae s.s.*, *An. funestus* and *An. arabiensis.* In controlling these vectors, ITNs act in three different ways; firstly, through provision of personal protection, by acting as a physical barrier between mosquitoes and the person sleeping under the net, secondly by reducing indoor biting by a combination of increased mosquito mortality, which is caused by the

insecticide on the net and the reduction of mosquito house entry caused by the nets excito-repellent properties (Lines et al. 1987, Lindsay et al. 1991). These properties combined lead to good protection (Lengeler 2004, Lengeler 2004) and an even bigger reduction in transmission, producing a community effect where high population coverage is achieved (Maxwell et al. 2002, Hawley et al. 2003, Killeen and Smith 2007, Le Menach et al. 2007). Indoor residual spraying works in the same way by, decreasing house entry and reducing the survival of the mosquitoes. It has a strong community effect, which contributes to reductions in malaria prevalence (Kouznetsov 1977, Mabaso et al. 2004, Nyarango et al. 2006, Kleinschmidt et al. 2007, Sharp et al. 2007). The greatest sustained success in Africa thus far achieved with IRS has been in South Africa (Mabaso et al. 2004), but growing resistance of malaria vectors to available insecticides like pyrethroids is a major cause for concern and an increasing threat to such essential and effective programs (Pages et al. 2007, N'guessan et al. 2007, Sharp et al. 2007).

Community-level effects which benefit unprotected individuals are attained by reducing the density, survival (Carnevale et al. 1988, Magesa et al. 1991, Robert and Carnevale 1991), human blood indices and feeding frequency of malaria vectors (Bogh et al. 1998, Charlwood et al. 2001). In reducing abundance and infectivity of malaria vectors, these tools reduce overall transmission and protect all individuals within a community (Lengeler 2004, Killeen et al. 2006), albeit with variation in responsiveness amongst vector populations. In this regard, the two interventions are not mutually exclusive (N'guessan et al. 2007). However, *An. gambiae s.s* and *An. funestus* are characteristically more amenable to control by IRS and ITNs than *An. arabiensis* due to its varied feeding and resting behaviour (Lengeler and Sharp 2003). In light of this inherent heterogeneity in the responsiveness of malaria vectors to control, these core interventions can be supplemented by larval source management strategies (e.g., larviciding and environmental management) in the context of integrated vector management (Utzinger et al. 2001, Killeen et al. 2002, Utzinger et al. 2002, Keiser et al. 2005, Townson et al. 2005).

While both IRS and ITNs remain the mainstay of malaria vector control (Protopopoff et al. 2008, Kleinschmidt et al. 2006, Lengeler 2004), the ownership and utilization of ITNs remains minimal in most endemic countries (Noor et al.

2009) and the operational deployment of IRS is more complex than ITNs. Deployment of these interventions together in high malaria risk areas is being advocated. Presently, there is mounting evidence that combining IRS and ITNs affords enhanced protection to exposed populations compared to using one method alone (Kleinschmidt et al. 2009). However, there are contradictory results from several studies that have compared IRS with ITNs for vector control with one method alone. Some studies have shown no positive combined effect of IRS and ITNs (Lengeler 2004, Protopopoff et al. 2008, Protopopoff et al. 2007, Nyarango et al. 2006) and others show incremental combined effect of IRS and ITNs compared with IRS alone (Rowland et al. 1997, Yadav et al. 1998, Lengeler 2004, Graves et al. 2008, Kleinschmidt et al. 2007). Although these two interventions have been critical in providing community protection the optimal policy for their co-implementation still remains to be determined.

Effective and sustained malaria vector control requires clear commitment from national authorities including long-term support from funding partners (Komatsu et al. 2010). Recently, there have been unprecedented increases in funding for malaria vector control in order to attain long-term goals of malaria elimination and global eradication (Feachem and Sabot 2008). Several malaria control programmes in Sub-Saharan Africa have fragmentary empirical evidence to inform policy formulation for rational vector control. As such, interventions are based on conventional assumptions such as: the rapid and significant impact of IRS in the short term for suppressing unstable malaria; the amenability of ITNs in effectively targeting the most vulnerable subgroups within communities with stable transmission; and, the greater operational and logistical ease of building and sustaining an ITN programme compared to an IRS one. For this reason, malaria control programmes are encouraged to adopt the WHO - led integrated vector management (IVM) strategy (Beier et al. 2008), which should be an evidence-based decision making process that requires a coherent monitoring and evaluation component (Van den Berg and Takken 2007). This should include routine surveillance of resistance profiles of major malaria vectors and potential resistance mechanisms to facilitate informed decisions and policy changes, such as the incorporation of insecticide resistance management operations into control programmes (Coleman et al. 2006, Hemingway et al. 1997).

1.5 Historical Development of Insecticides

Man has always had to cope with disease, discomfort and economic loss due to the presence of insect pests. With the view of improving health and socio-economic well being, methods to cope with human environmental demands were developed, amongst which was the emergency of pesticides for the control of insect pests responsible for both transmission of disease and for the destruction of crops (WHO 1957). Between 1867 and 1868, the scientific development and use of pesticides began with the use of the arsenical Paris Green and kerosene emulsions for spraying deciduous fruit trees. Following the discovery of botanical insecticides and the elucidation of their structure in the 1920s, their artificial synthesis commenced (Casida and Quistad 1998). In 1874 Zeidler first synthesized DDT and Paul Muller discovered its insecticidal properties in 1939. In 1943 DDT became the first insecticide to be commercially manufactured (Trigg and Kondrachine 1998). After the discovery and successful utilization of DDT, several other insecticides have been developed for use in public health, with further organochlorines and a carbamate being developed in 1945 and 1953 respectively (Brown 1978, Ware 2010, Casida and Quistad 1998).

1.5.1 Classification of Insecticides and Modes of Action

Insecticides are classified according to their chemical composition, origin, their toxicological action and their mode of penetration. In the later scheme, they are classified according to whether they take effect upon ingestion (stomach poisons), inhalation (fumigants), or upon penetration of the body covering (contact poisons). There are several classes of insecticides; however, those of public health significance can be divided into six major classes: organochlorines, organophosphates, carbamates, pyrethroids, insect growth regulators and microbial insecticides. The first four are the most used for public health chemical control and are briefly discussed below:

1.5.1.1 Organochlorines

The organochlorines are also referred to as chlorinated hydrocarbons, chlorinated organics, chlorinated insecticides, and chlorinated synthetics. There are four groups of organochlorines: diphenyl aliphatics, hexachlorocyclohexane (HCH),

cyclodienes, and polychloroterpenes. The insecticidal properties of HCH, synthesized in 1825, were discovered in 1942. It was used seldomly with the view of replacing DDT as resistance developed (Matsumura 1975). Cyclodienes; Aldrin and dieldrin were synthesized in 1948 and Chlordane in 1945. They are persistent insecticides, are stable in soil and relatively stable to the ultraviolet rays of sunlight. Unlike DDT and HCH, the cyclodienes have a positive temperature correlation: their toxicity increases with increases in temperature. Deldrin is more toxic than DDT and HCH to insects, human and animals, while less excito-repellent than DDT (Matsumura 1975). Dieldrin still remains useful for long-term use for inaccessible pest control treatments, such as termite control in house foundations. Toxaphene, synthesized in 1947 and strobane in 1951, are the only two polychloroterpenes insecticides (Matsumura 1975). Both are toxic to a wide variety of insects and are used in combating pests that attack cotton and other field crops. Toxaphene is readily degraded in the environment and only the metabolites hepta-, octa-1 and nonachlorobornanes accumulate in higher animals (Vetter and Scherer 1999).

DDT is the best known diphenyl aliphatic. Its mode of action is to disrupt axon depolarization of the sodium channel produced by inward sodium influx, which results from the activation and inactivation of the voltage-dependent sodium channel (Zlotkin 1999). DDT and its primary metabolite DDE are both stable and persistent in the environment and soluble in fat and insoluble in water (Carter 2004). While agricultural use of DDT has now ceased, due to environmental persistence and reduced efficacy against resistant insects, it is still extensively used for malaria vector control as a cost-effective and safe insecticide for indoor residual spraying.

1.5.1.2 Organophosphates

Organophosphorus insecticides (OPs) were discovered in 1854 but their insecticidal properties were only recognized by Schrader in 1937 (Matsumura 1975). The first organophosphorus insecticide to be developed was tetraethyl pyrophosphate (TEPP), used as a biological warfare agent in Germany during the Second World War (Casida and Quistad 1998). While OPs are among the most toxic insecticides to vertebrate animals, they are relatively more chemically unstable and non persistent in the environment than the chlorinated hydrocarbons. The first broad-spectrum insecticide in this group with minimal toxicity to mammals was malathion,

synthesized in 1950 (Matsumura 1975). Being esters or amides of organically bound phosphoric or pyrophosphoric acid, OPs can be divided into five classes according to their phosphorus moiety. However, those of agricultural and public health significance belong to only two classes, phosphorothioate insecticides (temephos, malation, pirimiphos, chloropyrifos, and fenitrothion) and phosphorothiolothioate (malathion) esters (Ware 2010).

Organophosphates act through inhibition of the normal function of acetylcholinesterase (AChE), which functions within the cholinergic nervous system by hydrolyzing the neurotransmitter, acetylcholine (Eto 1974). The enzyme is phosphorylated irreversibly by the insecticide (Cygler et al. 1993).

1.5.1.3 Carbamates

Carbamates are derivatives of carbamic acid that were originally extracted from the calabar bean in West Africa. The first carbamate insecticide, carbaryl, was introduced in 1956. The mode of action of carbamates is similar to that of OPs, inhibiting AChE by carbamylation. In insects, the effects of OPs and carbamates are primarily those of poisoning of the central nervous system, since the insect neuromascular junction is not cholinergic as in mammals. Carbamates, like OPs, are less persistent and more biodegradable in the environment than organochlorines. Propoxur and bendiocarb are the most commonly used insecticides in this group in malaria control programmes for indoor residual spraying, particularly in localities with DDT, OP and pyrethroid resistant vector populations. Propoxur was withdrawn in the early 2000's leaving bendiocarb as the sole carbamate available for vector control.

1.5.1.4 Pyrethroids

Pyrethroids, the newest generation of highly toxic insecticides of agriculture and public health significance, are synthetic derivatives of pyrethrum toxins. Pyrethrum was extracted from flowers of *Chrysanthemum cinerariaefolium* (Matsumura 1975, Ray 1991). The low toxicity and extreme photo liability of pyrethrins prompted the elucidation of the structure and synthesis of related pyrethroids with enhanced insecticidal properties and more stability to light and air (Hassal 1990). Increased attention and focus on pyrethroids for public health utilization followed in the wake

of the emergence of resistance to organochlorines, organophosphates and carbamate insecticides, coupled with their low volatility and polarity (which result in less movement in air or soil from the point of application). Presently the pyrethroids represent the most important insecticide class for the control of insects of medical significance. Deltamethrin, alphacypermethrin and lambdacyhalothrin are the most common pyrethroids used for malaria vector control.

All pyrethroids share a similar mode of action, acting on the sodium channel, in a manner similar to that of DDT and are considered axonic poisons. Pyrethroids affect both the peripheral and central nervous systems of the insect and work by keeping open the sodium channels in neuronal membranes. They initially stimulate nerve cells to produce repetitive discharges and eventually cause paralysis. The stimulating effect of pyrethroids is much more pronounced than that of DDT.

1.6 The Need for Chemical Control

Insecticides remain the most important element of integrated approaches to vector control. The recent restriction on the use of DDT by the Stockholm Convention on Persistent Organic Pollutants (POPs) has dramatically underlined the high degree of reliance of malaria control programmes on residual insecticides such as DDT (WHO 2001). To reduce this reliance, WHO is promoting integrated vector management, including alternative measures such as biological control or environmental management when and where they are effective and applicable. WHO also promotes the safe and targeted use of insecticides when there is no alternative (WHO 2004). For example, a very successful Chagas disease control programme in the Americas has been entirely based on indoor spraying of pyrethroid insecticides. Onchocerciasis (river blindness) has been successfully controlled for thirty years in eight countries of West Africa by weekly applications of a rotation of larvicides.

New technologies such as insecticide-treated bednets (ITNs) and insecticide-treated materials (ITMs) are now highly promoted and used to prevent diseases transmitted at night by mosquitoes and sandflies. Although applying insecticides on nets instead of walls is dramatically reducing the total amount of insecticide used for malaria prevention, ITNs remain highly dependent on a single class of insecticides; the

pyrethroids (WHOPES (b) 2007). Most insecticides belonging to other chemical groups do not have all the required attributes in terms of efficacy, speed of action and safety to be used on mosquito nets. The massive efforts currently developed to control malaria, especially in Africa, may be jeopardized by the widespread development of pyrethroid resistance (IRAC 2006).

1.6.1 The Threat of Insecticide Resistance

While insecticide application has performed a pivotal function in combating key disease vectors, excessive and indiscriminate utilization has exacerbated selection for insecticide resistance among the vectors they are intended to control (Hemingway and Bates 2003). Consequently, resistance to one or more insecticides of public health significance has developed in all major species of arthropod vectors such as mosquitoes, ticks, fleas, lice, and sand flies (Brogdon and McAllister 1988, Hemingway and Bates 2003); thereby precluding their control (Coleman and Hemingway 2007). Development of resistance is a complex and dynamic process and depends upon many factors. Most commonly, when the frequency of resistant insects in a vector population increases, efficacy of the treatment decreases up to the point where the insecticide has to be replaced by another one. Increasing the dosages in an attempt to maintain efficacy is not a recommended option because of environmental and safety concerns, increased cost of the insecticide and the resistance genes can be driven to even higher frequencies. Replacing an insecticide with a new one has important cost, logistic and sociological implications (IRAC 2006). A significant reduction of morbidity and mortality can be achieved only if the efficacy of vector-control interventions is continuously maintained at a very high level.

Almost all public health insecticides are also used in agriculture and high selection pressure has been ascribed to both agricultural and public health activities. The same insecticide classes are extensively used to control agricultural pests in Africa; this poses additional selection pressure on mosquitoes when insecticide contaminated ground water permeates their breeding sites (Ranson et al. 2009, Mouchet 1988, Lines 1988). Mouchet (1988) and Lines (1988) reviewed the link between the emergences of resistance with the expansion of agricultural activities. In Sri Lanka, resistance in one vector, *An. cuilicifacies*, is characteristic of public health spraying,

while resistance in the non-vector, *An. nigerrimus*, has a profile that indicates agricultural chemicals (Lines 1988). Equally, agricultural use of insecticides caused resistance in Central American *An. albimanus* (Rodriguez et al. 2006, Brogdon et al. 1988). Recently, development of vegetable farming has been associated with emergency of resistance to *An. gambiae* in urban areas of Benin (Yadouleton et al. 2009).

Moreover, many insecticides are also massively used to control domestic pests, and therefore, impact the vector species which are resting indoors. It is common for a single vector-mosquito population to be exposed to a given insecticide (e.g. a pyrethroid) at the larval stage through agricultural spraying and then again at the adult stage through household pest control, as well as vector-control programmes.

It is thus imperative to continuously monitor insecticide susceptibility and the underlying mechanisms responsible for the development of resistance to detect the early onset of resistance, and to predict the cross and multiple-resistance patterns that resistant species may exhibit from the type of mechanisms detected. This will support informed decisions making and policy formulation including the implementation of insecticide resistance management strategies to ensure sustainability (Coleman and Hemingway 2007). Ideally, an insecticide resistance monitoring program should cover a range of locations, including areas with historical or ongoing intense agricultural use of insecticides.

1.6.2 A Limited Number of Effective Insecticides

Although there is a relatively long list of public health insecticide products that can be used to control adult vectors, these products are all members of a small number of chemical groups with discrete modes of action. The list is further shortened by similarities in the mode of action across some of these chemical groups and the phenomenon of cross resistance. Cross-resistance explains why, in some situations, vector populations can develop resistance very rapidly to newly introduced insecticides. Furthermore, in some circumstances, resistance can persist in populations for very long periods after regular use of an insecticide has ceased. In these cases, resistance to new insecticides is inherited from the past as a result of the previous use of insecticides. Such situations reinforce the importance of: i)

understanding which target (s) insecticides are acting upon, and ii) precisely identifying the mechanisms involved once resistance has appeared in a vector population.

Only four different classes of insecticides play a significant role in public health for the control of mosquito adults; organophosphates (OP), organochlorides (OC), carbamates and pyrethroids (Coleman and Hemingway 2007). Since the introduction of these insecticides, selection pressure on vector populations has increased drastically (Nauen 2006). As a result, insecticide resistance has evolved to all four classes and over a hundred species of mosquitoes have become resistant to one or more insecticide (Hemingway and Ranson 2000). This poses enormous challenges to malaria control; pyrethroids are the only insecticides available for treatment of ITNs, there are restrictions on the number of insecticides suitable for IRS, and there are constraints imposed on insecticide choice by the insecticide profile of the targeted vector populations (Ranson et al. 2009, Coleman et al. 2006).

The resistance-related difficulties associated with vector-control efforts are exemplified by past attempts to control malaria. For example, DDT was first introduced for indoor residual spraying for mosquito control in 1946. The first cases of DDT resistance occurred in *Aedes* spp in 1947 (Brown 1986, Hemingway and Bates 2003). The resistance problems were exacerbated with the switch to newer insecticides, such as the organophosphates and pyrethroids. In 1955, the WHO called for the global eradication of malaria through the use of DDT (WHO 1957). However, emergence of DDT resistance (along with other logistical problems associated with deployment) derailed this effort and prompted the shift from malaria eradication to control in 1976 (Hemingway and Bates 2003). The status of pyrethroid resistance in anophelines has become worse since the late 1980s, and is on the increase. This is especially alarming in *An. gambiae* in Africa, where two different mutations - one originating in West Africa and one in East Africa - confer resistance (Malcom 1988, Chandre et al. 1999).

The detection of pyrethroid resistance of *An. funestus*, a vector amenable to control by both ITNs and IRS has implications for the malaria control programme (Brogdon and McAllister 1988, Casimiro et al. 2006). *An. funestus* is not DDT resistant in

most localities. The impact of public health spraying on development of resistance has been exemplified in Haiti, Sudan, Equatorial Guinea and Mozambique (Brogdon et al. 1988, Lines 1988, Sharp et al. 2007, Casimiro et al. 2006, Coleman et al. 2006).

1.7 Insecticide Resistance

Insecticide resistance is defined by the World Health Organization (WHO) as "the development of an ability in a strain of some organism to tolerate doses of a toxicant that would prove lethal to a majority of individuals in a normal population of the same species" (Zlotkin 1999, WHO 1957). Alternatively, a resistant phenotype has been defined as an insect that survives a dose of insecticide that would normally have killed it (Hemingway et al. 2002). This heritable change in the sensitivity of a vector population is reflected in the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that disease vector species (Nauen 2006).

The biological phenomenon develops as a result of selection pressure by the relevant insecticidal compound or its analogue (Bruce-Chwatt 1985). The level of resistance in insect populations is dependent on the amount and frequency of insecticides used, and the inherent characteristics of the insect species selected. Mosquitoes, for instance, are endowed with all attributes suited to rapid resistance development including high reproductive potentials and short life cycles producing several generations per season with abundant progeny (Bruce-Chwatt 1985, Nauen 2006).

Mostly resistance in a particular species is considered to occur throughout the control area, but in reality, insecticide resistance can be focal in nature and is very heterogeneous even over very small distances. It often develops within a small part of the population of one species of *Anopheles* and assumes different patterns depending on the type of selection pressure (Bruce-Chwatt 1985). In Guatemala, sampling sites for *Anopheles albimanus* only a few kilometers apart varied not only by presence or absence of resistance, but also by level of resistance and the mix of mechanisms responsible for resistance (Brogdon et al. 1988, Brogdon and McAllister 1988).

One of the four technical elements on which The Global Malaria Control Strategy is based is the need to strengthen local research capacities to enable the regular assessment of a country's malaria situation (WHO 1993). Today the major emphasis in research into vector resistance is double pronged. The first approach strives towards understanding the molecular mechanisms underlying resistance with the view of developing novel vector-control methods that avoid or minimize resistance problems. The second approach to research involves rational resistance management which is, developing and implementing control methods that minimize the likelihood that vectors will evolve strong resistance to important insecticides (Hemingway and Ranson 2000).

1.7.1 Genetic Evolution of Insecticide Resistance

The use of insecticides *per se* does not create resistance. Resistance occurs when naturally occurring genetic mutations allow a small proportion of the population (typically around 1 in 100 000 individuals) to resist and survive the effects of the insecticide. If this advantage is maintained by continually using the same insecticide, the resistant insects will reproduce and the genetic changes that confer resistance are transferred from parents to offspring, so that eventually they become numerous within the population. This "selection" process is the same as that which drives other evolutionary changes. The process will take longer if the gene conferring resistance is rare or present at a low frequency. Resistance should not be confused with tolerance that can occur after sub-lethal exposure to insecticide and is not passed on to offspring.

A fundamental principal in population genetics is the Hardy-Weinberg law that describes and predicts genotype and allele frequencies in a non-evolving population, under the assumptions that: 1) the population is large (i.e., there is no genetic drift); 2) there is no gene flow between populations, from migration or transfer of gametes; 3) mutations are negligible; 4) individuals are mating randomly; and 5) natural selection is not operating on the population. Given these assumptions, a population's genotype and allele frequencies will remain unchanged over successive generations, and the population is said to be in Hardy-Weinberg equilibrium. The Hardy-Weinberg model can also be applied to the genotype frequency of a single gene. The model enables us to compare a population's actual genetic structure over time with the genetic structure we would expect if the population were in Hardy-Weinberg

equilibrium (i.e., not evolving). If genotype frequencies differ from those we would expect under equilibrium, we can assume that one or more of the model's assumptions are being violated, and attempt to determine which one(s).

Natural populations with whole genotypes in Hardy-Weinberg equilibrium are rarely found; one or more of the assumptions are violated in most situations. If nothing else, most populations are under the influence of natural selection. Certainly no population can be infinite, but many populations are not even large enough to be functionally infinite. Often populations are not completely isolated from one another, and migration of individuals into or out of one population can change its genetic makeup. Mutations can potentially alter the gene pool significantly, although the majority is thought to have little or no effect (neutral mutations). Finally, individuals often mate selectively rather than randomly.

1.7.2 Major Factors that Influence Resistance Development

Insecticide resistance is a multidimensional biological phenomenon that depends for its development on the interaction of multiple influences (WHO 1957). The multiple factors that influence the development of resistance to insecticides can be categorised into five groups:

1.7.2.1 Frequency of Insecticide Application

How often an insecticide or control tactic is used is one of the most important factors. With each use, an advantage is given to the resistant insects within a population. The rate of increase of resistance on any population will be faster in the presence of a lower fitness cost.

1.7.2.2 Dosage and Persistence of Effect

The duration of effect or persistence of an insecticide is affected by the physical chemistry of the insecticide, the type of formulation, and the application rate. Products which provide a persistent effect can be considered to act in a similar manner to multiple treatments in that they provide continual selection pressure. For example, a space spray will persist for a very short time and will select only against a single generation of mosquitoes. In contrast, a residual wall application or an ITN treatment will persist for months or years and therefore can potentially select against

many generations of the same insect. It is therefore important to always follow WHO or manufacturer recommendations and to use products at full recommended rates.

1.7.2.3 Rate of Reproduction

The number of generations produced per year, and the rate of increase and fluctuations in population size are critical. Insects that have a short life-cycle and high rates of reproduction are likely to develop resistance more rapidly than species which have a lower rate of reproduction because more generations and more insects may be rapidly exposed to an insecticide application. Mosquitoes have a history of insecticide resistance and are characterized by a relatively short life-cycle and high fecundity, with females laying several hundred eggs during their reproductive life. In contrast, the tsetse fly does not typically resist insecticides and has a longer life-cycle and relatively low rate of reproduction, with females producing in total fewer than 10 larvae.

1.7.2.4 Population Isolation

Behavioural attributes like migration in and out of exposed populations, and response to repellent effects of insecticides are also potential contributors. With disease vectors, the goal is often to eliminate all or most of the population, but the more selection pressure that is put on a population, the faster resistance will evolve. The immigration of individuals possessing susceptible alleles from untreated areas will beneficially dilute and compete with resistant-insect alleles in treated areas. An early step in vector-control programmes should therefore be to identify the source of the vectors and to estimate the significance of immigration of untreated insects. For instance, an island where the entire area was receiving treatment would be at very high risk of developing resistance. Awareness of, and coordination with, other vector-control programmes and agricultural activities should occur so that the regional effect on the target population is considered.

1.7.2.5 Genetic

Mutation rates and frequency of resistant (R) allele/s in wild populations, penetrance, expressivities and dominance of R genes, and relative fitness of the genotypes influence resistance development. Resistance genes can range from

dominant through semi-dominant to recessive. If dominant or semi-dominant, only one parent must possess the trait for it to be fully or partially expressed in the offspring. If recessive, both parents must possess the trait. Fortunately, most resistance mechanisms (for example *kdr*) are controlled by recessive or semi-dominant genes, which increases the chance of managing resistant populations. If the resistance is genetically dominant, it can rapidly become established within the population and be difficult to manage.

1.7.3 Dominance Levels and Insecticide Resistance

While the frequency of the R gene/s and the past selection from other insecticides have obvious implications for vector control, phenotypic expression of R alleles relative fitness of the genotypes allude to the evolution of insecticide resistance in a wild population based on the comparative dominance of the resistance allele (Curtis and Davidson 1981, Hemingway 1981, Rawlings et al. 1981). Where the frequency of the R allele is low it exists almost exclusively in the heterozygous state. The level of dominance is a measure of the relative position of the heterozygous phenotype relative to the phenotype of the two corresponding homozygous (Bourguet et al. 1996). If a wild type gene (A) mutates to a deleterious allele (a), the Aa heterozygote often displays a wild-type phenotype: the deleterious effects of mutations are fully recessive. This depends on where the mutation is and the normal function of the mutated gene.

Initially, the dominance level was determined by comparing the mortality curves of homozygous susceptible, resistant and heterozygous individuals (Milani 1963). Before the quantitative measure of dominance level was introduced by Stone (1968) resistance was qualitatively classed as recessive or dominant according to whether the heterozygote mortality curve was closer to the homozygote susceptible or resistant mortality curve, respectively and co-dominant if the heterozygote mortality curve was equidistant from those of the homozygotes.

It is an established principle in population genetics that a rare recessive gene will increase in frequency under selection less rapidly than a rare dominant gene. For example, a population under insecticide selection pressure carrying a rare dominant R allele will constantly be forwarding heterozygous individuals to succeeding generations. The R allele frequency will increase slowly under these conditions.

Alternatively, an R allele that is phenotypically recessive in expression will increase in frequency rapidly owing to exclusive selection of RR homozygotes. R alleles of intermediate dominance will fill the spectrum between these two extremes.

Insecticide resistance provides a good model to study dominance relationships (Bourguet et al. 2000) because many of the genes and mutations responsible for resistance have been identified, and the physiological processes in which the resistance genes are involved are known. Additionally, there is a large variation of the level of dominance of resistance, eg. the insecticide resistance phenotype conferred by mutations decreasing the affinity of insecticide target sites varies from complete recessivity to complete dominance. A resistance allele may be dominant (over a susceptible allele) for one species and recessive for another.

When discussing the issue of dominance of a resistance allele, the environmental parameters should always be specified, as dominance describes the relationship between the phenotypes of the three genotypes, which may vary between traits and environments (Marinez-Ramirez et al. 1995). Dominance can be determined through different means based on:

- The position of the mortality curve for heterozygous individuals relative to those for both homozygotes, at a given mortality level (Bourguet et al. 1996).
- The mortality of heterozygous individuals relative to that of both homozygotes, at a given insecticide concentration (Roush and Mckenzie 1987, Curtis 1978).
- A comparison of the fitness of the heterozygotes relative to that of the two homozygotes at a given insecticide dose (Bourguet et al. 1996).

Dominance level models suggest that this spectrum covers a range of 5 to 50 generations of selection for a significant degree of resistance to appear (May 1985) and takes into account migration of susceptible alleles so that selection for resistance alleles and migration of susceptible alleles act as antagonistic forces in a finite environment (Naglaki 1975). This principle, coupled with the fitness costs associated with most resistance genotypes, allows for migration distribution and

fitness costs of resistance whereby precise knowledge of migration distribution and fitness costs of resistance enable one to calculate a critical area of insecticide application below which resistance alleles should fall to extinction (Lenormand et al. 1999).

1.7.4 Fitness Cost

In population genetics the term fitness means "success in producing offspring, irrespective of the causes of that success" (Paul 1992). Fitness cost should be understood as "the decrease in an organism's ability to survive and reproduce in other environments following a mutation that confers selective advantage in one environment". Populations of insects that have never been exposed to insecticides are usually fully susceptible, and resistance genes within those populations are very rare. This is usually due to a "fitness cost", which means that insects possessing the resistance gene lack some other attribute or quality, such that it gives an advantage to the susceptible insects in the absence of the insecticide. Differences in the number of offspring, longevity or overall robustness can often be measured in resistant insects. There is good laboratory and field evidence to suggest that the absence of selection pressure (in the form of insecticide treatment) in most cases operates against resistance. Resistant colonies in the laboratory often revert to susceptibility if the insecticide selection pressure is not maintained. Similarly once resistance in the field has been selected it often reverts once the insecticide treatment regime is changed. A good example of this occurred in *An. arabiensis* in Sudan, where malathion-specific insecticide resistance was selected in the early 1980s through antimalarial house spraying. The development of resistance prompted a switch of insecticide treatment to fenitrothion and the malathion resistance rapidly reverted over the next few years (IRAC 2006).

It is this reversion to susceptibility which is the underlying assumption behind any effective resistance-management strategy. However, reversion rates are potentially variable and may be very slow, particularly when an insecticide has been used for many years. If there is no fitness cost for the resistance there is no reason for the resistance genes to be lost in the population and for resistance to fully revert. For example, DDT was used extensively for malaria control over a 20-year period up to the 1960s in Sri Lanka to control *An. culicifacies* and *An. subpictus*. DDT was

replaced by malathion in Sri Lanka in the early 1970s when a total and effective ban on DDT use was implemented. Subsequent regular monitoring has shown that DDT resistance has reverted very slowly towards susceptibility. Around 80% of the adult mosquito population was resistant in the 1970s compared to about 50% in the 1990s. This rate of reversion is clearly too slow to establish any effective resistance-management strategy involving the reintroduction of DDT.

1.7.5 Methods of Quantifying Insecticide Resistance

Information on insecticide resistance is important to inform effective vector control policy formulation (Coleman and Hemingway 2007). As such, detection and monitoring of insecticide resistance in malaria vectors is crucial, and has to be conducted together with other entomological surveys. An overview of insecticide resistance detection and monitoring activities has been summarized by Brent, (1986) as follows:

- Check for the presence and frequency of occurrence of expressed resistance genes in target organism population.
- Gain early warning that the frequency of resistance is rising and/or that practical resistance problems are starting to develop.
- Determine the effectiveness of management strategies introduced to avoid or delay resistance problems.
- Diagnose whether rumoured or observed fluctuations or losses in the field efficacy of a chemical are associated with resistance.
- If resistance has been confirmed, determine subsequent changes in its incidence, distribution, and severity.
- Give practical guidance on pesticide selection in local areas.
- Gain scientific knowledge of the behavior of resistant organisms in the field in relation to genetic, epidemiological and management factors.

Establishing an effective resistance management plan requires simple assays to monitor and evaluate insecticide resistance and its underlying mechanisms. To this end, different biochemical and molecular approaches have been devised, some of which are amenable for field operations. The contemporary applicable methods for

resistance monitoring of field populations of mosquitoes are outlined below.

1.7.5.1 WHO Diagnostic Assays

A bioassay is used to determine the relationship between a physiologically active agent and the effect that it produces in a living organism (Hoskins and Craig 1962). Bioassays with the dosage or the exposure time as the variable are carried out to test the resistance status of insect populations. The lethal dosage (LD), lethal concentration (LC) or lethal time (LT), which kills 50% ($LD_{50}/LC_{50}/LT_{50}$) or 90% ($LD_{90}/LC_{90}/LT_{90}$) of the population can be calculated from such bioassay data (Matsumura 1975) and compared with a known susceptible population of the same species, after which resistance is expressed in relative terms.

The WHO diagnostic assay is the most widely used technique in the field. The candidate insects are exposed to filter papers impregnated with insecticide in carrier oil formulation. The insecticide discriminating dosage is set at twice the LD_{99} that kills 100% of three day old non-blood fed adult females of known homozygote susceptible laboratory colonies for a range of Anopheline mosquitoes (WHO 1963, WHO 1980). While this is a robust dosage that susceptible individuals are unlikely to survive by chance, and WHO guidelines suggest that resistance is only indicated if more than 5% of insects survive the exposure, reducing the risk of false positives, many heterozygous resistant individuals may be killed by the dose. Hence resistance levels may need to be very high before they are detected using this methodology (Coleman and Hemingway 2007).

A current list of recommended diagnostic dosages for many insecticides for a number of arthropod disease vectors is published by WHO, and impregnated papers and test kits can be bought from a centralized distribution centre in Penang (WHO 1995, W.H.O 1998).

These assays (with the potential exception of dieldrin bioassays) cannot be used to accurately monitor resistance gene frequencies in mosquito populations. They also give no indication of the underlying mechanisms of resistance, and hence they have no predictive value for anticipating cross-resistance between insecticides (Coleman and Hemingway 2007). Therefore, the resistance status detected using bioassays,

can then be further studied by looking at the mechanisms responsible for resistance using biochemical and molecular assays.

1.7.5.2 Synergists

Synergists are enzyme inhibitors of insecticide detoxification enzymes. The synergists, piperonyl butoxide (PBO) and S, S, S- tributylphosphorotrithioate (DEF) are inhibitors of monooxygenases and esterases respectively (Devine and Denhom 1998, Soderlung et al. 1990). Glutathione s-transferase activity is inhibited by Ethacrynic acid (EA), diethyl maleate (DM), and chlorfenethol (CF). By inhibiting specific detoxification enzymes, insecticide synergists can reduce or eliminate the selective advantage of individuals possessing over-expressed or mutated enzymes (Matsumura 1975). Therefore, they are used to suggest the type of metabolic resistance mechanisms present in insect populations (Scott et al. 1990). For example, they are used in bioassays to counteract or inhibit the enzymes responsible for resistance to the insecticide. Some are used in control to reduce the dose or rate of application (Devine and Denhom 1998). For example, piperonyl butoxide is commonly added to pyrethroid-based aerosol formulations to decrease the time to knock down and increase the time to recovery from the insecticide.

1.7.5.3 CDC Bottle Assay

These are similar to the WHO discriminating dose assays. However, the CDC bottle assay relies on time mortality data, which are measures of the time it takes an insecticide to penetrate a vector, traverse its intervening tissues, get to the target site, and act on that site. Insecticide impregnated bottles are prepared by coating glass bottles with an acetone or alcohol based formulation. Insects are then exposed to the insecticides in the bottles. This assay has the advantage over the WHO test kit that the rate of insecticide knock down is easier to score during the course of the exposure period. With rapid acting insecticides, such as pyrethroids this can be predictive of a *kdr*-type resistance mechanism within the population, although care should be taken not to over-interpret such data, as several effective metabolic resistance mechanisms also produce a reduced knock-down phenotype without any accompanying change in sensitivity at the sodium channel target site (Brogdon and McAllister 1988).

Bioassay data generated by either the CDC or WHO method is a good indicator of the presence of resistance in mosquito populations, but they cannot be used to measure gene frequency accurately or suggest the epidemiological impact of resistance. Resistance gene frequencies in general will be higher than indicated by bioassay data alone (Casimiro et al. 2007). Hence bioassays are not sufficiently sensitive to monitor low level resistance. A central theme of all resistance management modeling is that resistance needs to be detected at very low frequencies; hence methods that facilitate measuring the frequency of different mechanisms of resistance in field populations of mosquitoes are required. Methods currently available vary in their sophistication and ease of use (Coleman and Hemingway 2007).

1.7.5.4 Biochemical Assays

There are two ways that metabolic enzymes can produce resistance (Hemingway 1981); overproduction of the enzyme, which leads to either increased metabolism or sequestration of the insecticide and an alteration in the catalytic centre activity of the enzyme, which increases the rate of insecticide metabolism by the enzyme. Sequestration occurs when the overproduced enzyme rapidly binds and slowly metabolizes the insecticide, therefore preventing it from reaching the target site within the insecticide (Aldridge 1993). With sequestration the resistance level is proportional to the increase in the quantity of the enzyme produced because of the slow insecticide turn-over rate (Aldridge 1993). Biochemical assays are used to give a first indication of the enzyme system involved in resistance (Hemingway 1981). A number of simple biochemical assays (W.H.O 2000) are available to detect increased activity of three enzyme systems, esterases (Brogdon et al. 1988, Dary et al. 1990, Brengues et al. 2003), GST (Brogdon and Barber 1990) and P450's (Brogdon et al. 1997) involved in insecticide metabolism. Many of these assays detect increased enzymatic activity against model substrates in resistant individuals.

One of the most common metabolic resistance mechanisms in Culicine mosquitoes involves gene amplification of one or more esterase that sequester organophosphates and slowly turns them over. Initial methods for elevated esterase detection were filter paper-based (Dary et al. 1990), having the advantage of producing permanent records of results, but the method had the disadvantage that esterase, and hence

resistance levels were not easily quantifiable. Later methods were microtitre plate based, allowing accurate quantification of esterase levels with access to a plate reader, although results could still be scored by eye as with the filter paper tests. Biochemical assays for the GSTs and P450's are less-field applicable. The GST microtitre plate assay, although accurate, requires access to a plate reader able to measure absorbance at 340 nm. There is no direct assay for P450 activity in individual insects. A modified haem assay allows a very crude estimation of the amount of P450 present in single insects, but results are difficult to interpret, detecting only very high levels of enzyme elevation.

Only one of the target sites, acetylcholinesterase (AChE) is amenable to development of a biochemical detection system (Hemingway et al. 1986). A simple microtitre plate assay is available to measure AChE insensitivity using a carbamate or an oxon analogue of a phosphorothioate insecticide. In contrast to all the other metabolic microtitre plate assays, this assay is sufficiently accurate to measure resistance gene frequencies, allowing differentiation between homozygous and heterozygous resistant individuals.

1.7.5.5 Molecular Assays

Molecular techniques can be used to detect some well characterized resistance mechanisms. Most techniques employ the method of Polymerase Chain Reaction (PCR). Mutations in the insecticides target site lend themselves to detection through simple PCR assays, which can readily be used in many field settings. Allele specific PCR assays have been developed for three major target sites, the GABA receptors (ffrench-Constant et al. 1994, Du et al. 2005), the sodium channels (*kdr*) (Martinez-Torres et al. 1998, Lynd et al. 2005) and AChE. The challenge is to adapt these assays for high throughput field application, as they have the advantage of detecting heterozygous resistant individuals that may be missed by other assays (Coleman and Hemingway 2007).

All enzymes involved in detoxifying insecticides belong to large enzyme families, members of which have varying substrate specificities. In many cases of insecticide resistance which have a metabolic basis, the exact molecular mechanism of resistance is unknown; hence allele specific assays are not yet available. However,

recent advances in genomics have allowed a much more rapid identification of genes that are up or down regulated in insecticide resistant insects using microarray technology (David et al. 2005). The detoxification microarray chip, developed for *An. gambiae*, contains all potential insecticide resistance genes. Population screening using this chip has allowed the rapid identification of genes that are up or down regulated in resistant compared to susceptible insects. These differentially regulated genes are being expressed to directly assess their ability to metabolize or bind insecticides. The availability of this technique has reduced the time required to fully document the resistance genes within a population from years to months. Positional cloning approaches have then confirmed the co-location of these up-regulated genes with the physical location of the insecticide resistance quantitative trait loci. Once resistance genes have been identified they can then be screened for allele specific single nucleotide polymorphisms (SNPs) that segregate with the resistance phenotype and the SNPs will then form the basis of a simple PCR type assay that can be used routinely in field populations. Combining the high technology approach of microarrays with routine population monitoring with simple PCR technology will afford a better way of accurately monitoring the frequency of known resistance genes in field populations of mosquitoes (Hargreaves et al. 2000). This technology also has the added advantage over bioassays that it can be undertaken on dead mosquitoes. The biochemical assays, while they can be undertaken on dead mosquitoes, require that the mosquitoes have not had immediate prior exposure to insecticides and that the mosquitoes have been preserved by freezing after death (Coleman and Hemingway 2007).

The detoxification chip can also be used to screen for resistance genes in other *Anopheles* species. Screens have already been successfully undertaken on *An. stephensi* and work is underway on pyrethroid resistant *An. funestus*. A similar detoxification chip has been developed for *A. aegypti* and currently a large number of potential insecticide resistant strains are being screened to identify the major metabolic resistance genes in this species, which is the major dengue vector.

1.8 Mechanisms of Insecticide Resistance

Although resistance arises through Darwinian selection in a population, it is often a combination of factors, that results in the overall expression of resistance (Bruce-Chwatt 1985). Physiological resistance, for example, arises through various mechanisms, viz; reduced penetration (of the insecticide through the cuticle), site insensitivity (i.e. the target site is altered and not affected by the insecticide), increased metabolic detoxification (so that it is detoxified before it reaches the target site), sequestration (i.e. stored in the body where it is not harmful) and possible increased excretion. Reduced penetration involves changes that decrease the rate of penetration of insecticide through the insect cuticle and confers low levels of resistance (Hemingway 1981, Oppenoorth 1985). Whereas behaviour involves changes resulting in reduced contact with insecticide (Sparks et al. 1989), this is characteristically difficult to quantify (Miller and Salgado 1985).

Insecticides from the four public health pesticide classes are nerve poisons and either target acetylcholinesterase in the synapses or voltage-gated sodium channel on the insect nerves (Ranson et al. 2009). The molecular basis of insecticide resistance has been attributed to the existence of mutations in target site genes or metabolic alterations at the level of the activity of the detoxification proteins (Brogdon and McAllister 1988, Hemingway 2004). Insecticide resistance mechanisms have a biochemical basis (Brogdon and McAllister 1988) and target-site resistance and detoxification enzyme-based resistance remain the two major forms of biochemical resistance (Nauen 2006, Brogdon and McAllister 1988).

1.8.1 Metabolic Resistance

Three key enzyme groups: a) esterases, b) oxidases and c) GST are responsible for metabolic detoxification based resistance to organochlorines, OPs, carbamates, and pyrethroids (Hemingway and Bates 2003, Brogdon and McAllister 1988, Clark and Shamaan 1984), although none of them are unique to resistant insects. Resistance can be due to a structural change in the enzyme molecules that increase its ability to detoxify the insecticide and/or an increase in the amount of the enzymes produced and thus preventing the insecticide from reaching its site of action (Nauen 2006, Brogdon and McAllister 1988).

1.8.1.1 Monooxygenase-Based Resistance

The monooxygenases are a complex family of oxidative enzymes involved in the metabolism of xenobiotics. Enzymes associated with enhanced oxidative metabolism are the cytochrome P450-dependent mixed function oxidases (MFOs) or microsomal monooxygenases (P450's). At least four (families 4, 6, 9, 18) of cytochrome P450s have been isolated from insects (Ranson et al. 2002, Brogdon and McAllister 1988). The enzymes responsible for resistance , which, like the esterases, occur in Diptera as a cluster of genes (Tomita and Scott 1995). The P450 monooxygenases have overlapping substrate specificities (Brogdon and McAllister 1988, Wilkinson 1976). P450s, therefore, confer resistance to all insecticides primarily to pyrethroids and carbamates, and a lesser extent to organochlorines and organophosphates (Coleman and Hemingway 2007). Elevated monooxygenase activity is associated with pyrethroid resistance in *An. stephensi*, *An. gambiae* (Vulule et al. 1994), and *Culex quinquefasciatus* (Kasai et al. 1998) and *An. funestus* (Wondji et al. 2009). Enhanced levels of monooxygenases in resistant insects result from constitutive over expression rather than amplification (Tomita and Scott 1995, Carino et al. 1994). They are also responsible for activating the phosphorothioate insecticides to their active oxon analogues. P450 enzymes bind molecular oxygen and receive electrons from NADPH to introduce an oxygen molecule into the substrate. Products of the oxidative process are more water-soluble and, are easier to excrete.

1.8.1.2 Esterase-Based Resistance

The esterase-based resistance mechanisms involve modified levels or activities of esterase detoxification enzymes that metabolize insecticides like organophosphates, carbamates and pyrethroids by hydrolysis of ester linkages. These esterases comprise six families of proteins belonging to the α/ß hydrolase fold superfamily (Cygler et al. 1993, Oakeshott et al. 1993). In Diptera, they occur as a gene cluster on the same chromosome (Campbell et al. 1997, Russell et al. 1996, Newcomb et al. 1997, Hemingway and Bates 2003). Esterases are important in resistance to organophosphate and carbamate insecticides and to a lesser extent pyrethroids (Kadous et al. 1983). These enzymes have been studied extensively in the mosquito *C. quinquefasciatus,* where increased levels of one or more esterases, due to gene

amplification, are responsible for broad-spectrum organophosphate resistance (Vaughan and Hemingway 1995). In several Culicine species the esterases act by rapidly binding and slowly turning over the insecticide: They sequester rather than metabolize the insecticides (Vaughan et al. 1997). In contrast, in a number of *Anopheles,* a malathion non-elevated carboxylesterase-type mechanism produces resistance through increased metabolism of the insecticide (Herath and Davidson 1981, Herath et al. 1981, Hemingway 1982, Hemingway 1983, Hemingway 1985, Malcolm and Boddington 1989). In contrast to pest insects of agricultural importance, esterases have not yet been shown to play a major role in conferring pyrethroid resistance in mosquitoes (Nauen 2006).

1.8.1.3 Glutathione S-Transferase-Based Resistance

GSTs are multifunctional enzymes responsible for the detoxification of a variety of xenobiotics (Hemingway and Bates 2003, Brogdon and McAllister 1988). The enzymes catalyse the nucleophilic attack of reduced glutathione (GSH) on the electrophilic centres of lipophilic compounds. Multiple forms of GSTs have been described in most insects from at least three families (Ranson et al. 2002, Hemingway and Bates 2003, Hayes and Pulford 1995) and have a role in insecticide resistance. These enzymes are primarily involved in DDT, pyrethroid and organophosphate resistance. In mosquitoes, two families of GSTs are elevated in DDT resistant insects (Grant and Matsumura 1989, Grant and Hammock 1992, Prapanthadara et al. 1993). DDT detoxification involves a dehydrochlorination process, which results in its conversion to DDE, a less toxic isomer. Elevated GST levels have been studied in *An. gambiae* and *An. dirus*, where resistance is primarily due to changes in the regulation of one or more GST families (Enayati et al. 2005).

1.8.2 Target-Site Resistance

This form of biochemical resistance occurs when the insecticide no longer binds to its target. The organophosphates, carbamates, organochlorines, and pyrethroids all target the nervous system (Nauen 2006, Hemingway and Bates 2003). However, alterations of amino acids responsible for insecticide binding at its site of action cause the insecticide to be less effective or even ineffective (Brogdon and McAllister 1988). There are three types of resistance involving site insensitivity; altered acetylcholinesterase, reduced neuronal sensitivity to chlorinated cyclodienes

and reduced neuronal sensitivity to DDT and pyrethroids. Alterations have been observed in neuronal enzymes and receptors, which are the target site of the majority of insecticides used in vector control.

The three major target sites for current public health insecticides are:

- Acetylcholinesterase (AChE), which breaks down the neurotransmitter acetylcholine.
- Ligand-gated ion channels (Rdl) that bind chemical signals, such as λ-aminobutyric acid (GABA), which is then converted into electrical signals via the opening of their integral ion channels.
- Voltage-gated channels, such as the sodium channel that are triggered by changes in membrane voltage rather than changes in the concentration of a neurotransmitter.

1.8.2.1 Acetylcholinesterase (AChE)

The target of organophosphorus (OPs) (e.g., malathion, fenitrothion) and carbamate (e.g., propoxur, bendiocarb) insecticides is acetylcholinesterase (AChE) in nerve cell synapses. AChE, a serine esterase that hydrolyses the excitatory neurotransmitter acetylcholine is found on the post-synaptic nerve membrane. Alterations in AChE in organophosphate and carbamate resistant insects result in a decreased sensitivity to insecticide inhibition of the enzyme by these insecticides (Ayad and Georghiou 1975, Hemingway and Georghiou 1983). The OPs are converted to their oxon analogues, via the action of monooxygenases before acting as AChE inhibitors. Insensitive acetylcholinesterase (iAChE) has been documented in resistant strains of several insect, tick, and mite species. By itself, iAChE tends to provide different levels of resistance to different insecticides but this can range from 2-100-fold (Brown 1986). Should AChE be inhibited by insecticide, acetylcholine continues to facilitate synaptic after-discharges causing insect paralysis or death (Hemingway and Karunaratne 1998).

1.8.2.2 GABA Receptors

The GABA receptor in insects is a gated chloride-ion channel, a widespread inhibitory neurotransmission channel in the central nervous system and in neuromuscular junctions (Bermudez et al. 1991, Hemingway and Bates 2003). It is a site of action for pyrethroids and avermectins as well as cyclodienes (Kadous et al. 1983, Bloomquist 1994). Dieldrin resistance is conferred by a single nucleotide change within the same codon of a gene for a γ- aminobutyric acid (GABA) receptor (ffrench-Constant et al. 1994). The mode of action of cyclodienes is to block the inhibitory action of the neurotransmitter receptor γ-butyric acid (GABA). GABA facilitates the uptake of chloride ions causing hyper-polarisation of the chloride ion channel. Inhibition of GABA results in hyper-excitation.

1.8.2.3 Sodium Channels

The sodium channels of the nerve sheath are the target of organochlorines (DDT) and pyrethroids (Brogdon and McAllister 1988). DDT and pyrethroids cause persistent activation of the sodium channels by delaying the normal voltage-dependent mechanism of inactivation (Soderlund and Bloomquist 1989). The *para*-gated sodium channel is governed by a single point mutation in the knock down resistance (*kdr*) gene coding for the target site resulting in reduced knockdown and lethal effects of DDT and pyrethroids. As this is the target site of DDT and pyrethroids, this mechanism produces cross-resistance to the two insecticide classes by single amino acid substitutions (one or both of two known sites) in the axonal *para*-gated sodium channel gene (Miyazaki et al. 1996, Williamson et al. 1996). Such a mutation is under pressure from two sources: the mutation must confer resistance and it must not interfere with the normal function of the target protein. In *An. gambiae* two mutations, Leu-Phe (Martinez-Torres et al. 1998) in West Africa and Leu-Ser (Ranson et al. 2000) in East Africa have been identified at the same codon.

1.8.3 Cross and Multiple Resistance

Cross-resistance occurs when a resistance mechanism, that allows insects to resist one insecticide, also confers resistance to compounds within the same class, and may occur between chemical classes (depending on mechanism). The phenomenon of cross-resistance is a relatively frequent one in vector populations. For example, DDT and pyrethroid insecticides are chemically unrelated, but both act on the same

target site (sodium channel). Past use of DDT has resulted in several insect species developing resistance to DDT by the *kdr* mutation at the target site. Where these mutations have been retained in the population, the insects have some resistance to all pyrethroids in addition to DDT. Cross-resistance can also occur between OP and carbamate insecticides when resistance results from an altered AChE (Daly et al. 1998). Cross resistance depends on the operational relationships of chemicals in use to insecticides used earlier, and level of insecticide exposure.

Multiple resistance is a common phenomenon and occurs when several different resistance mechanisms are present simultaneously in resistant insects. The different resistance mechanisms may combine to provide resistance to multiple classes of products. It is also quite common for the contribution of different mechanisms to change over time as selection processes evolve (Daly et al. 1998).

1.9 Insecticide Resistance Management

The long term control of vectors is threatened by insecticide resistance which is occurring at a faster pace than new insecticides are being developed. This can be exemplified by the dramatic impact of pyrethroid resistance to the malaria vector *An. funestus* in southern Africa (Maharaj et al. 2005), which was correlated to a dramatic increase in malaria incidence in the region. The historical response of waiting until a shift has occurred in an epidemiological end point for disease to assess if vector control has failed, is no longer sustainable, insecticide resistance management is essential to conserve scarce public health insecticides (Hemingway et al. 1997).

With only four classes of insecticides recommended for the control of adult mosquitoes it is vital that effective resistance management strategies are employed. Resistance management entails the development and implementation of control interventions that minimize the likelihood that vectors will evolve strong resistance to important insecticides (Georghiou 1994). The aim is to prevent or delay the onset of resistance in populations exposed to an insecticide, or develop management programs that cause existing resistance in populations to decline, through rotating or alternating insecticides as a resistance management strategy before resistance

reaches measurable levels (Curtis et al. 1993, Hemingway and Bates 2003). Resistance surveillance is a fundamental step and insecticide susceptibility an indispensable resource of resistance management (National Research Council, 1986). Resistance surveillance has three objectives: 1) To provide baseline data for program planning and insecticide selection before the commencement of control operations; 2) To detect resistance at an early stage so that timely management can be implemented; 3) To continuously monitor the effect of control strategies on resistance.

Resistance management can be defined as "the containment of the frequency of resistance genes below an acceptable threshold by means of strategic choices of insecticide, dosage, mode of application, or frequency of use" (Georghiou 1994). Resistance management strategies take advantage of the adverse fitness costs of resistance genes, to the insects carrying them, in the absence of insecticide selection pressure. Random genetic events generate mutant alleles some of which confer insecticide resistance. Alleles with strong pleiotropic effects are generally selected against in the absence of selection pressure. When insecticide selection pressure is applied, the frequency of resistant alleles increases. The dominance status of the trait is important as this can affect the outcome of resistance management strategies.

Computer models have identified some key factors affecting the evolution of resistance (Boete and Koella 2003, Mani 1985, Raymond et al. 1998). These models provide a simple means of predicting the efficacy of different management strategies. However, models often lack critical information such as population size, migration rates, selection intensity and the fitness of alleles. This lack of information undermines the models (Hemingway et al. 1997, Coleman and Hemingway 2007). Several models have been tested under laboratory conditions, but rarely in field conditions. Most resistance management models assume that resistance to insecticides is monogenic and independent of resistance to other insecticide classes (Tabashnik 1989) and work on the following assumptions:

- Resistance is controlled primarily by a single-gene locus with two alleles, R (resistant) and S (suseptible), with a fixed dose-mortality line for each genotype.

- The dose-mortality line for RS heterozygotes is intermediate between the SS (susceptible) and the RR (resistant) lines. At low pesticide doses RS heterozygotes are not killed, and the R gene is effectively dominant; at high doses RS heterozygotes are killed, and the R gene is effectively recessive.
- The insect life cycle is divided into sub stages, with transition probabilities between sub stages determined by natural and pesticide mortalities.
- Immigrants are primarily susceptible and have at least one day to mate and reproduce before being killed by a pesticide.

Utilizing the tools currently available for monitoring insecticide resistance trials in southern Mexico compared changes in frequencies of resistant alleles under mosaic and rotational resistant management strategies to single insecticide use (Hemingway et al. 1997).

Using a single insecticide assumes that the initial frequency of the resistant allele is low and the vast majority exist as heterozygotes. Applying an insecticide at a dose rate that is sufficiently high to kill all heterozygotes has been advocated. The frequency of homozygote resistant mosquitoes is assumed to be so low they would be overwhelmed and mate with homozygous susceptible immigrant mosquitoes. For this approach to succeed all heterozygous individuals must receive the appropriate lethal dose. This is difficult to achieve under field conditions and is costly with environmental implications (Curtis and Davidson 1981).

An alternative is to utilize a mixture of two or more insecticides, the aim being that resistance will evolve more slowly to both insecticides. Mixtures of insecticides require the expected frequency of resistant alleles at two different genetic loci to be low and that individual mosquitoes carrying both alleles are rare (Curtis and Lines 1985). Using two or more insecticides in a spatial pattern assumes that individual mosquitoes will be exposed to more than one insecticide. Hence a mosaic should have similar results to insecticide mixtures. The effects of mixtures in delaying the development of resistance have been evaluated in the laboratory with *C. quinquefasciatus* (Lagunes Tejeda 1980), *Musca domestica* (Pimentel and Bellotti 1976), and in the field with citrus thrips (Immaraju et al. 1990).

Temporal rotation where insecticides are applied in an alternating sequence is also based on the assumption that an individual mosquito does not carry two resistant alleles (Georghiou 1980). Should the frequency of an allele increase in a population under selection by an insecticide, they will be killed when the switch is made to the next insecticide. It is assumed that the resistant gene will have a selective disadvantage during the absence of selection pressure. If this assumption does not hold true, rotation of insecticides will not prevent the accumulation of resistant alleles. The rotational strategy has been explored using models and computer simulations (Comins 1986).

There are few long term field studies on the resistance gene frequencies to asses the fitness costs in natural populations. The common prediction of models is that resistance will reduce without selection pressure. This has been demonstrated in the mosquito *Culex pipiens* (Yebakima et al. 2004) and sheep blow fly *Lucilia cuprina* (Kotze and Sales 2001).

There is a renewed interest in integrated vector management approaches that encompass larviciding using biological larvicides and environmental management (Fillinger et al. 2008, Geissbuhler et al. 2007, Mukabana et al. 2006, Chanda et al. 2008, Killeen et al. 2004, WHO 2004) with a view of reducing selection pressure. There is also need for comprehensive knowledge and understanding of the malaria vector species distribution, abundance, infectivity and behaviour and the human reservoir infectiousness. To fight malaria effectively, such baseline knowledge is critical in understanding their role in malaria transmission and hence its control as well as monitoring and evaluation of the effects of control methods including surveillance of insecticide resistance in vector species (Coleman and Hemingway 2007, Okara et al. 2010).

1.10 Malaria Transmission and Evaluation of Vector Control Interventions

The survival and bionomics of all arthropod vectors of disease is affected by climate change, particularly rainfall, temperature and relative humidity (Hay et al. 1996). The rise in the earth's temperature of between 0.3^{o}C and 0.6^{o}C witnessed last century impacted adversely on most vector-borne diseases, including malaria (Lindsay and Birley 1996). Minute spatial variations and temporal heterogeneities in the mosquito population can have important consequences for disease transmission (Lindsay and Birley 1996, Cattani et al. 2005, Smith et al. 1994, Sharp and le Sueur D. 1996). Climate variability has resulted in changes in malaria endemicities globally (Nchinda 1998) particularly drought (Lindsay and Birley 1996) and global warming (Connor et al. 1997) and hence affecting malaria transmission by its impact on the sporogonic cycle (n) and mosquito survival (p) in accordance with the MacDonald (1957) basic reproduction rate (McMichael and Haines 1997, Sharp and le Sueur D. 1996).

Malaria transmission involves a complex interaction between vector, host, parasite and the environment; and is governed by different ecological determinants (Hay et al. 1996) including local factors such as socio-economic, socio-demographic, socio-cultural and behavioural patterns of the community (Daash et al. 2009). Transmission of malaria is effected by the exposure of the human host to blood-feeding infectious *Anopheles* mosquito vectors. The mosquito is infectious when the sporozoites released from mature oocysts are present in the salivary gland of the mosquito (Baton and Ranford-Cartwright 2005). Sporozoite-stage parasites inoculated by even a single infectious mosquito can cause human malaria infection and life threatening disease (Beier et al. 1994, Trape and Rogier 1996). To fight malaria successfully, control programmes must use current tools effectively and measure the impact of these tools on transmission (Shaukat et al. 2010).

The implementation of an effective and evidence-based vector control strategy requires locally informed decisions because the epidemiology of malaria varies at a small scale, suggesting the need for precise targeting (Van den Berg and Takken 2007). As the disease has several biological and environmental determinants there is a need for integrated monitoring, evaluation and continuous surveillance. Cognizant

of the heterogeneities in operational settings, the WHO has set recommendations to guide effective deployment of interventions in both high and low transmission epidemiological settings (WHO 2009, WHO 2008). The intensity of malaria transmission affects most aspects of malaria epidemiology and control (Snow et al. 1997, Snow and Marsh 2002, Struik and Riley 2004, Reyburn et al. 2005).

The intensity of malaria transmission can be measured in several ways: Parasite rate, annual parasite index, spleen rate and the entomological inoculation rate (EIR) (Killeen et al. 2002, Warrell and Gillies 2002, Fontenille and Simard 2004, Killeen et al. 2000, Smith et al. 2009, Smith and McKenzie 2004, Smith et al. 2007). Most of these indices, derived from field and theoretical data, are calculated using assumptions and are generally not used for evaluating control programmes (Shaukat et al. 2010). Good estimates of malaria transmission intensity are therefore necessary to compare and interpret malaria interventions conducted in different places and times and to objectively evaluate options for malaria control (Snow et al. 1997).

While IVM is a conceptual strategy other than a physical one, it is a decision making process for management of vector populations to reduce or interrupt transmission. One of the key features of IVM is capacity building at the operational level to plan, implement and monitor and evaluate vector control and its epidemiological and entomological impact (WHO 2004). Monitoring of determinants affecting both transmission and infectious reservoir of the parasite is critical in determining the impact of interventions through continuous surveillance. Most determinants relating to the vector; density, vectorial capacity and resistance can be influenced by programme interventions. Human factors affecting contact with the vectors are population density, movement, proximity to vectors, domestic conditions and practices. Environmental determinants comprise the climate and ecosystem, land use and availability and location of alternate hosts (Van den Berg and Takken 2007).

Monitoring and evaluation, which are critical elements of effective vector control, have two inter-related components: 1) monitoring and evaluation of programmatic implementation (process) and 2) monitoring and evaluation of interventions (Outcome and Impact). Impact measures the reduction observed in transmission of

the disease through defined indicators whose calculation is based on epidemiological and entomological surveillance (WHO 2003). A basic understanding of relationships between malaria transmission by the vector mosquito and disease outcomes in measuring transmission is essential. The epidemiological impacts expected from any entomological intervention are a reduction in parasite prevalence, incidence, morbidity and mortality (Githeko 2006, Beier et al. 1999). The entomological correlates of epidemiological impacts are vectorial capacity, entomological inoculation rates and the basic reproductive number (R_0) all of which have a bearing on the vector species abundance and infectivity (Smith et al. 2007, Githeko 2006).

The basic reproductive number, R_0, is an important concept that has played a central role in epidemiological theory for malaria and other infectious diseases because it provides an index of transmission intensity and establishes threshold criteria. R_0 is generally defined as the expected number of hosts who would be infected after one generation of the parasite by a single infectious person who had been introduced into an otherwise naive population (Anderson and May 1991, Dietz 1993). If $R_0 > 1$, the number of people infected by the parasite increases, and disease persists, with the level of transmission depending on the size of R_0. If $R_0 < 1$, the number declines and consequently the disease decreases and will eventually disappears from the population (Smith et al. 2007, Silver 2008). Thus, if sustained disease control reduces transmission intensity by a factor that exceeds R_0, the parasite will eventually be eliminated. Alternatively, the fraction of a population that would need to be protected to confer ‘‘herd immunity’’ and interrupt transmission is $1 - 1/R_0$. R_0 represents the maximum reproductive rate per generation, leaving aside complications such as host immunity and super infection (Smith et al. 2007).

Vectorial capacity is the entomological component of the basic reproduction rate of malaria. It is defined as the future daily sporozoite inoculation rate arising from a currently infected human case, on the assumption that all female mosquitoes biting that person become infected. Reducing vectorial capacity reduces R_0. It is the product of the vector density in relation to man, the proportion that bites man twice, and the expectation of the infective life span of the vector (Macdonald 1957, Garrett-Jones and Shidrawi 1969). Vectorial capacity is mathematically expressed as:

$$VC = Ma^2 p^x / - \ln p$$

where, M = man-biting rate or vector density in relation to man, a = the daily man–biting rate, p = daily survival rate, x = duration of the sporogonic cycle. Expectation of the life span of a vector = 1/-log p, and Expectation of the infective life span = p^x/-log p. However, vectorial capacity is the indirect method of estimating transmission rate by a malaria vector.

The entomological inoculation rate (EIR), also known as infective biting rate (IBR), or the inoculation rate (h) remains the most direct measurement of malaria parasite transmission intensity. It is used to assess the effect of anti-vector interventions, as the tools currently considered as able to interrupt malaria transmission. It quantifies the parasite-infected mosquito pool and its propensity to transmit infectious parasites to the human population (Shaukat et al. 2010). Therefore, malaria transmission intensity is best expressed as the EIR, which directly reflects the exposure of humans to pathogenic *Plasmodium* parasites (Killeen et al. 2000, Beier et al. 1999).

The EIR is the number of infectious bites per person per unit time, usually measured or expressed per year. It can be estimated as the product of the human reservoir infectiousness (k), the life-time transmission potential of individual mosquitoes (L) and the rate at which they emerge from larval breeding sites (E) relative to human population size (E/N_h) (Killeen et al. 2000):

$$EIR = k\ L\ E/N_h$$

Alternatively, EIR can be expressed as a product of the human biting rate and the sporozoite rate:

$$EIR = MaS$$

The human biting rate (*Ma*) is the number of vectors biting an individual over a fixed period of time. *M* equals the number of *Anopheles* per person and *a* equals the average number of persons bitten by one *Anopheles* in one day. The sporozoite rate (S) is the fraction of vector mosquitoes present and biting that are considered infectious, i.e. *Anopheles* with sporozoites in their salivary glands (Warrell and Gillies 2002, Snow and Marsh 2002). The structure of the EIR equation directly implies that measures which reduce the value of any of these contributors will amplify each other's effects when combined and thus decrease the EIR. These three contributors are also discreet targets for transmission control that are reduced by

quite different interventions (Killeen et al. 2000). The only intervention envisioned which could usefully reduce k, and which is likely to be available in the foreseeable future, is a malaria vaccine (Miller and Hoffman 1998) and widespread use of transmission-blocking drugs. Tools for the reduction of L include indoor residual spraying, insecticide treated bed nets, and zooprophylaxis (Snow et al. 1999, Rozendaal 1997, Lengeler et al. 1998) whereas source reduction and other forms of larval control represent well established methods for controlling E/N_h (Kitron and Spielman 1989, Soper and Wilson 1943, Shousha 1948).

The EIR values are harnessed for the quantification of the impact of available vector control tools; IRS, ITNs and source reduction (SR) on malaria transmission. Numerous factors influence EIR, including temperature, altitude, rainfall and urbanization (Warrell and Gillies 2002). In general, the EIR is directly proportional to temperature because heat accelerates the sporogonic cycle, the time necessary for ingested gametocytes to develop into infectious sporozoites. The optimal temperature for malaria transmission is 25-27ºC and an average monthly relative humidity of about 60% (Pampana 1969). For the same reason, the EIR is inversely proportional to altitude because temperature decreases as altitude increases. The EIR is directly proportional to rainfall because female *Anopheles* lay eggs in fresh water. Generally, the EIR is inversely proportional to urbanization because with urbanization comes fewer bodies of water and greater pollution of water sources (Robert et al. 2003). Therefore, tropical areas with warm temperature, heavy rainfall, high humidity, and efficient *Anopheles* vectors are ideal for malaria transmission (Breman et al. 2001). These factors explain a large part of the variability in the EIRs across Africa.

An adult mosquito's lifespan is particularly important in the transmission of malaria. The mosquito must survive long enough for the parasite to complete sporogonic development from the point where gametocytes are ingested with the blood meal to the time when infectious sporozoites appear in the salivary glands. This process typically takes 10 days for *P. falciparum* (Killeen et al. 2002). Therefore, decreasing the life span of mosquitoes substantially decreases the EIR.

In Africa, many studies have demonstrated that standard vector control measures are

useful for controlling and even eliminating malaria in certain areas where transmission levels are marginal (Mouchet 1998). A foundation of malaria vector control is that actions to decrease vector-host contact through methods including larval habitat modification, insecticide treatment of larval habitats, spraying inside of houses with residual insecticides, insecticide-treated bed nets, or the use of repellents will have corresponding beneficial outcomes in terms of reduction in morbidity and mortality. Effective vector control measures the incidence of malaria infections because there is a linear relationship between EIR and malaria incidence (Beier et al. 1994). Studies in Saradidi in western Kenya have showed that 74% of the variation in *P. falciparum* incidence is explained by EIR (Beier et al. 1994).

The prediction of malaria transmission intensity in the form of an EIR is more useful than either vectorial capacity or reproductive number because this parameter is a better epidemiologic predictor and can be measured directly (Killeen et al. 2000). An EIR below one is needed to interrupt malaria transmission (Shaukat et al. 2010), but many errors in EIR estimation can occur due to the difference in both the human biting rate and sporozoite rate that result from variation in methods used, subjectivity of mosquitoes to the capturer, and diligence of the technical teams (Fontenille and Simard 2004). The lack of consistently used standard EIR protocols, including logistical difficulties and ethical issues concerning human landing catches mean that the use of EIR by control programmes is greatly undermined. Several methods are used to measure the human biting rate, including using "captures" (human landing catches), pyrethrum spray catches, exit trap collections, and CDC light traps (W.H.O 1975). Human landing catches are the gold standard proxy of human-biting rates, but the logistical difficulties coupled with ethical issues undermine its use in most malaria endemic areas.

IVM involves a "rational decision-making process for the optimal use of resources for vector control" (Beier et al. 2008). It requires reconsidering the combination of vector control methods over time, as the environment, epidemiology, and resources change (Shaukat et al. 2010). The use of two or more vector control methods in the context of the IVM strategy may be a more effective way of reducing malaria transmission, if each method targets a setting most susceptible to that intervention. Thus, impacts of more than one of these interventions will amplify each other's

effects (Killeen et al. 2000). Although IRS, ITNs, and SR are all effective individually, they complement each other and can have a synergistic impact when used together (Okech et al. 2008). The model by Killeen et al (2000) predicts that ITNs and IRS are the most effective tools available for reducing EIR; source reduction amplifies the results. Therefore, an integrated vector approach can meaningfully reduce EIR and larval control amplifies the effect of adult vector control.

Due to heterogeneity in transmission, it is necessary to realistically describe the inter-relations between *P. falciparum* parasite rate, entomological inoculation rate and the basic reproductive number and the newer malaria transmission models do this (Smith et al. 2005). However, whether these sources of heterogeneity can be practically identified, mapped, and targeted to maximize the effect of interventions, remains a challenge (Hay et al. 2008). A large remaining topic for research is identifying the human and vector-based contributions to this transmission heterogeneity (Smith et al. 2005). However, measurable impacts of specific intervention measures on the vector population, sporozoite rates or infectious reservoir have been observed in the field, as alternatives to EIR (Beier et al. 1999, Shaukat et al. 2010, Charlwood et al. 1998, Molineaux 1997, Saul 1993, Killeen et al. 2000, Macdonald 1957).

Although parasitaemia, i.e. the presence of malaria parasites in blood films from peripheral circulation, counted in 100 high power fields (Klinkenberg et al. 2006), has been increasingly used as a proxy of impact of malaria interventions (Shiff et al. 1996, Curtis et al. 1998, Menendez et al. 1997, Lengeler et al. 1995, D'Alessandro et al. 1995, Korenromp et al. 2004, WHO 2005), by routinely monitoring vector species density and infectivity it is possible to measure the direct effect that the vector control programme is having on transmission of malaria (Cuamba et al. 2006, Kleinschmidt et al. 2006, Sharp et al. 2007). Although sporozoites indicate that a species is a vector, the sporozoite rate varies within a species relative to vector control interventions and the vectors insecticide resistance status. In Angola, for example, the sporozoite rate was 1.9% for *An. gambiae* and 0.7% for *An. funestus* (Bigoga et al. 2007, Cuamba et al. 2006). In another study in Bioko Island, the first IRS round with a pyrethroid had no effect on the number of *An. gambiae s.s.*

collected, but reduced their sporozoite rate, thereby substantially lowering their transmission potential. Prior to the first round of IRS, sporozoite rates were 6.0, 8.3 and 4.0 for *An. gambiae s.s.*, *An. melas* and *An. funestus* respectively showing *An. melas* to be an important vector in areas in which it occurred. After three spray rounds, no infective mosquitoes were identified (Sharp et al. 2007).

Insecticide resistance surveillance informs decision and policy making and allows incorporation of insecticide resistance management operations into control programmes (Coleman and Hemingway 2007, Hemingway et al. 1997, Sharp et al. 2007). In areas with pyrethroid-susceptible *An. gambiae*, there has been no detectable difference in the efficacy of IRS versus ITNs (Curtis and Townson 1998). However, vector densities have been shown to vary tremendously with insecticide resistance status (Sharp et al. 2007). The operational significance of insecticide resistance for malaria control has been demonstrated in Mozambique and Bioko Island (Kleinschmidt et al. 2006, Sharp et al. 2007). After using a pyrethroid the number of *An. gambiae s.s.* were not reduced due to resistance but *An funestus* population declined from 23.5 to 3.1 per trap per 100 nights. After the introduction of a carbamate insecticide , *An. gambiae s.s.* reduced from 25.5 to 1.9 per trap per 100 nights (Sharp et al. 2007). Hence, a thorough appreciation of resistance profiles of major malaria vectors assisted informed decisions and policy changes.

While the effectiveness of IRS and ITNs, including their comparative operational impact upon malaria transmission, has been demonstrated (Lengeler and Sharp 2003, Neville et al. 1996, Roberts 1964, Curtis et al. 1999), there is mounting evidence that a combination of both strategies confers an additive protective effect (Pardo et al. 2006, Nyarango et al. 2006, Beier et al. 2008, Kleinschmidt et al. 2009). Nevertheless, most assessments of impact of IRS and ITNs on malaria transmission have been conducted in high transmission areas rather than in low transmission areas (Roberts 1964, Curtis et al. 1999, Goodman et al. 2001, Guyatt et al. 2002, Protopopoff et al. 2007, Protopopoff et al. 2008, Beier et al. 2008). In areas at low risk of malaria infection, such as South Africa and the high lands of East Africa, IRS has proved to be a highly effective protective intervention (Roberts 1964, Curtis et al. 1999). For example, IRS reduced transmission by 75%, compared to 63% for ITNs in Kenya (Guyatt et al. 2002). However, in another low

transmission area of Kwa Zulu-Natal in South Africa, ITNs were more effective (Goodman et al. 2001). In a high transmission area of Tanzania the effectiveness of the two interventions was equivalent (Curtis et al. 1999, Curtis et al. 1998) and implementation of both IRS and ITNs reduced malaria infection rates by 50% in the Island of Bioko in Equitorial Guinea (Pardo et al. 2006).

While operational and implementation research to inform policy decisions should be a prerequisite in defining the type of interventions suitable for the local settings such as optimal use of LLINs and IRS, or when intervention coverage levels can be reduced, the use of such monitoring and evaluation by most programmes has been anecdotal. Currently interventions are primarily being monitored through population based surveys, with a bias to the infectious reservoir of the parasite, malaria specific mortality and all cause mortality. Surveillance and epidemic preparedness systems should be well entrenched in malaria control programmes as a means of reducing morbidity rates and case fatality (Nyarango et al. 2006). The NMCP should collect routine surveillance data on biological and the environmental determinants of malaria disease: parasite, vector, human and environment, and continuously analyze the data and results fed into the national data base to facilitate decision-making and policy formulation. Because these interventions are currently being scaled-up by many malaria control programmes, it is critical to optimize the measurements of their impact on disease transmission and guide decision making and policy formulation.

1.11 The Malaria Situation in Zambia

1.11.1 Malaria Disease Burden

In Zambia malaria is the leading cause of morbidity and mortality accounting for 40% of outpatient attendances, 45% of hospital admissions with 47% and 50% of disease burden among pregnant women, and children under-five years of age respectively. Case fatality rates among hospital admissions are estimated at 40 per 1,000 (MoH 2007, Chanda et al. 2009). Current trends in the country indicate that malaria is responsible for at least 3 million clinical cases and about 6,000 recorded deaths annually, including up to 40% of the under five deaths and 20% of maternal mortality (MoH 2007, Chanda et al. 2009). It is anticipated that the actual morbidity

and mortality may be far higher if unreported cases and community deaths are included. Over the past three decades, Zambia experienced an exponential increase in the malaria burden. The malaria incidence tripled from 121.5 cases per thousand in 1976 to about 394 cases per thousand in 2003 (MoH 2006). Malaria incidence per 1,000 population increased from 394 to 428 in 2004. Cases increased by 21% from 3.6 million to 4.3 million, while the deaths declined from 8,952 to 8,289 or by 7%. Many factors led to this increase. These included the spread of drug resistance, reduced vector control, decreased access to health care, HIV/AIDS and poverty (MoH 2006). The disease accounts for the greatest number of Disability Adjusted Life Years (DALYS) lost (6.8 million) followed by the Acute Respiratory Infections (5.4 million) and HIV/AIDS (3.2 million) (MoH 2000).

The malaria transmission levels in Zambia are driven by *An. gambiae s.s, An. funestus and An. arabiensis* across the country. While malaria distribution is not uniform, the disease is endemic in all nine provinces of Zambia and is hyper-endemic in hot riverine valleys with perennial transmission, meso-to hypo-endemic on plateaus, and hypo-endemic in urban areas. Three epidemiological strata for malaria have been identified; (i) the riverine and basin areas, (ii) the high land and plateau areas and (iii) the urban areas. In terms of transmission potential again the country can be divided into three regions or belts; (a) the northern belt (b) a central belt (c) a southern belt. It is estimated that 100% of the population is at risk of exposure to malaria. Malaria transmission, influenced by annual precipitation levels, altitude and related temperatures, is all year round, with the peak occurring during rainy season from November to April. Thus incidences of malaria are high in the years when the country receives higher than average rainfall.

1.11.2 Historical Malaria Control Efforts

The history of malaria control efforts in Zambia dates back to 1929 at the Roan Antelope Copper mine in Luanshya (Watson 1953, Utzinger et al. 2001), and has progressed through several stages (Table 1.1). Pioneering interventions constituted environmental management and mosquito net use, coupled with diagnosis and treatment using quinine (Utzinger et al. 2002). This stimulated entomological studies on malaria vector bionomics (DeMeillon 1937, Adams 1940, Pielou 1947, Paterson 1963) to further guide deployment of interventions.

Table 1.1: Milestones in the History of Malaria Vector Control in Zambia: 1929 to 2010

1929	Inception of malaria prevention and control efforts in Zambia
1932	Malaria legislation
1937	De Meillon research on vector behaviour (*An. gambiae* complex)
1944	Enactment of the Mosquito Extermination Act (environmental management)
1947	IRHS the Federal Malaria Eradication Programme in urban areas
1963	Split of Federation, Northern Rhodesia begins to lose resources to Southern Rhodesia
1964	Amendment of Mosquito Extermination Act (measures to reduce mosquito breeding)
1973	IRHS coverage in urban areas reduces to 30% and vector studies by Shelly
1975	Chemoprophylaxis introduced in rural areas
1979	Studies on vector bionomics by Bransby Williams
1980	Mines reduced expenditure on malaria control
1985	UNICEF funded ITN project initiated in Samfya district
1992	Health reforms and inclusion of malaria in the basic health care package
1994	JICA funded ITN project in Chongwe district
1995	Annual in vivo surveillance commenced by NMCP, documentation of rising resistance to chloroquine, WHO funded ITN funded project in Ndola
1997	Zambia signs the WHO AIM Harare Declaration, USAID and JICA funded Eastern Province Integrated Malaria Initiative in three districts
1998	Extensive Malaria KAP studies
1999	Malariometric surveys to define endemicity and consolidation of the ITN distribution through the Community based Malaria Prevention and Control programme in 41 districts
2000	Development of the first 2000-2005 National Malaria Strategic Plan, reintroduction of IRS by the private-sector and prioritization of ITNs for vector control by the malaria control programme
2001	Consultative discussions by the public sector with private sector and other stakeholders on IRS
2002	Needs assessments for IRS implementation conducted in 5 districts and Introduction of multiple ITN distribution mechanisms
2003	Treatment policy change and reintroduction of IRS by the Public sector
2004	Introduction of the Integrated Vector Management strategy, scaling up IRS to 8 districts and the waiving of taxes and tariffs on ITNs and retreatment kits by the government
2005	Development of the current 2006 – 2010 National Malaria Strategic Plan, strengthening of supervision, geo-coding and logistics for IRS by HSSP, SEA conducted in 15 IRS districts and introduction of the free mass distribution of ITNs in Zambia. Environmental management for malaria control launched in Lusaka on 21st October 2005
2006	Rapid scale up of ITNs for impact covering 6 of the 9 provinces in the country and consultative meeting with Valent Biosciences Coorporation on larval source management using Bio-larvicides.
2007	Sockage pits, wash bays and evaporation tanks constructed in 15 districts, efficacy studies on larvicides (*Bacillus thuringesis var.israelensis*, Insecticide Growth Regulators and Monomolecular Surface Films) conducted by the National Malaria Control Centre
2008	Public sector scales up IRS to 36 districts, Production of guidelines on distribution and utilization of ITNs for Malaria Prevention and Control, Feasibility assessments for integrating LSM into the malaria control programme by Durham University, VBC and WHO conducted in Lusaka, Position statement on LSM made and Larviciding piloted in the urban areas of the initial 5 IRS districts, An inter-sectoral stakeholders consensus meeting on scaling up LSM to urban areas of 8 districts held on 22nd January at Edinburgh Hotel in Kitwe
2009	Production of country specific guidelines for IRS in Zambia and scaling up the mass distribution of ITN to all the nine provinces, The use of larvivorus fish (*Gambusia affinis*) launched on 25th April during the commemoration of the World Malaria Day, Needs assessments for scaled up LSM implementation conducted in eight urban districts. Commodities and equipment procured using GFATM Round 4 and distributed, and implementation funds disbursed to eight districts
2010	IRS scaled up to 54 districts, training and orientation of community and district health management teams on LSM and Implementation in May 2010, Monitoring and Supervision conducted in collaboration with Konkola Copper Mines and Mopani Copper Mines

The success of the urban malaria control was enhanced by the enactment of the statutory instrument "the Mosquito Extermination ACT" in 1944 (MoJ 1944) that was later amended in 1964 (MoJ 1964) to oblige householders, mining companies, irrigation and water utility works to undertake specific measures to stop mosquito

breeding. By 1950s, Indoor Residual Spraying (IRS) with DDT was adopted in urban communities, with further reduction of the malaria incidence and the disease became notifiable in the Copper belt and the city of Lusaka (MoH 2000). However, by 1973, IRS coverage was reduced by 30% due to economic constraints and environmental concerns over DDT use, and finally stopped in the mid 1980s. The reduced vector control levels, coupled with development of parasite resistance to anti-malarials (Himpoo and MacCallum 1967, Kofi Ekue et al. 1983) led to an upsurge of malaria cases (Utzinger et al. 2002).

While Zambia has a history of successful malaria control through the late 1970s (Utzinger et al. 2001, Utzinger et al. 2002, Watson 1953), inequities in deployment of prevention and control strategies have been demonstrated. The urban areas benefited from the IRS with DDT. The rural areas were using chemoprophylaxis, which was introduced in 1975, with chloroquine for school children, under five children and pregnant women (MoH 2000). This reduced the urban malaria burden significantly while the rural populace continued to suffer a huge burden. Municipal councils were responsible for the urban areas, whilst the Ministry of Health was responsible for the rural areas. Developmental projects, particularly in the mining areas, were responsible for control in their areas.

1.11.3 Malaria Control Policy Change

As Zambia began health reforms in 1992, control of malaria was included in the basic health care package as a priority disease (MoH 2000). Challenges facing malaria control included; shifting from parasite control to the management of disease using rational and sustainable methods with a focus on prevention through vector control; access to quality health care and the introduction of user fees that followed in the wake of decentralization. Malaria KAP studies and malariometric surveys were conducted in 1998 and 1999 respectively (MoH 2000).

The establishment of the Roll Back Malaria (RBM) partnership stimulated the development of the 2001-2005 national malaria strategic plan (NMSP) that emphasized malaria case treatment with sulphadoxine-pyrimethamine and chloroquine and disease prevention through deployment of ITNs (MoH 2001). However, due to increase in anti-malarial drug resistance (Barat et al. 1998, Bijl et

al. 2000) the drug policy was changed to ACTs in 2003 (Sipilanyambe et al. 2008). In 2000, Konkola Copper Mines (KCM), a private company, reintroduced IRS with pyrethroids and DDT in two mining districts (Sharp et al. 2002). The success of these IRS programmes led the NMCP to include IRS in its arsenal.

Malaria remains as a public health priority in Zambia, which is emphasized in both the 2006-2010 fifth National Development Plan and the 2005-2009 National Health Strategic Plan (MoH 2006). In 2005, the Ministry of Health (MoH) developed a 2006-2010 NMSP with the vision *"A malaria free Zambia*". The main goal for malaria control is "*to reduce malaria incidence by 75% and under-five mortality due to malaria by 20% by the year 2010"* (MoH 2006). This policy included an effective drug policy with ACTs (Sipilanyambe et al. 2008, MoH 2006) and scale up of definitive diagnosis using microscopy and RDTs. Vector control was scaled up using ITNs (Figure 1.3) and IRS (Figure 1.4) with some supplementary larviciding using *Bacillus thuringensis* var. *israelensis* (Bti). The strategic interventions also include: intermittent presumptive treatment for pregnant women, research, monitoring and evaluation, and behaviour change communication. The implementation of an integrated malaria control strategy by the Ministry of Health through the national malaria control programme has resulted in remarkable reduction in the disease burden in the country (WHO 2009).

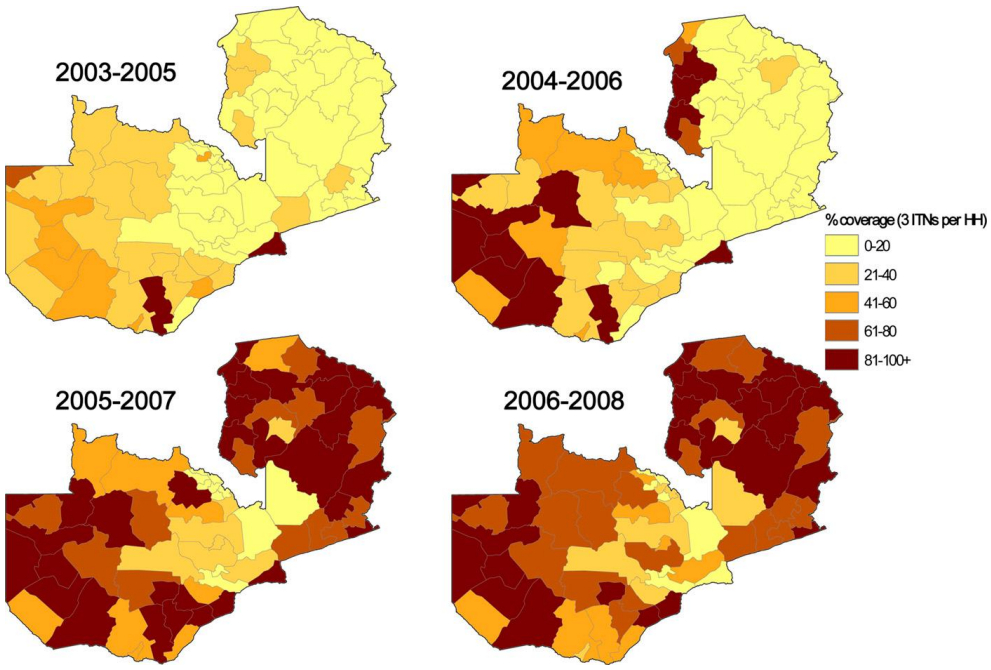


Figure 1.3: Estimated operational coverage of 3 insecticide-treated mosquito nets (ITNs) per household in overlapping 3-year intervals based on ITN distributions by district in Zambia from 2003–2008.

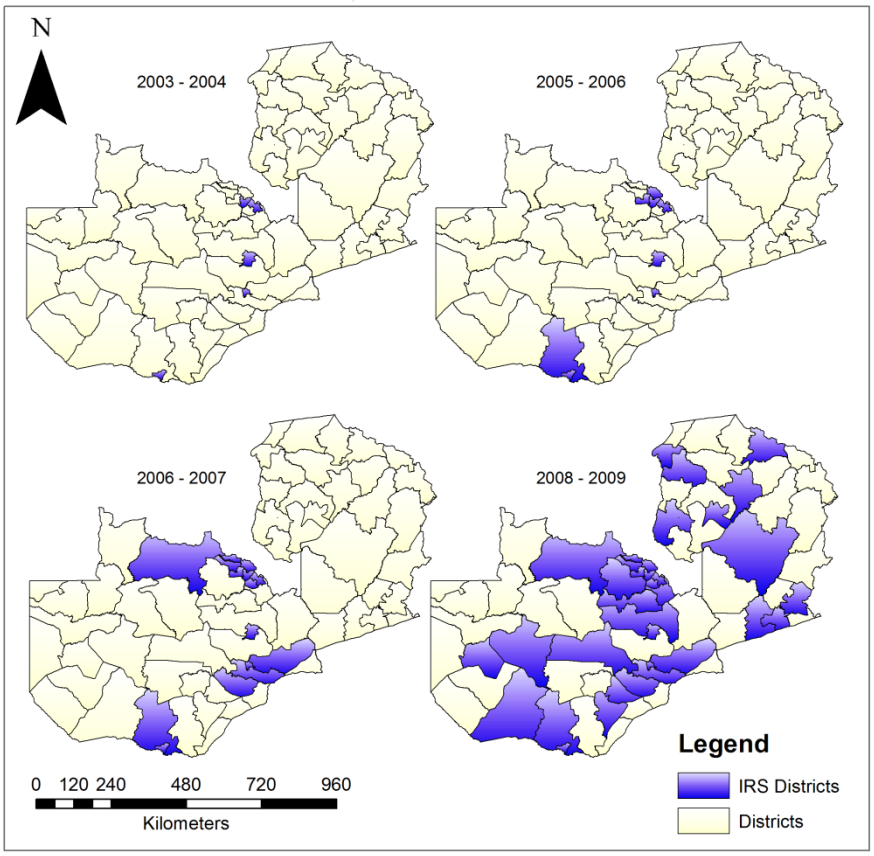


Figure 1.4: Operational coverage of 36 indoor residual spraying (IRS) districts in Zambia from 2003–2008.

1.11.4 Malaria Vector Species Composition in Zambia

The malaria vector system in Zambia belongs to the Southern African eco-epidemiological strata of the Afro-tropical zoogeographical region (Macdonald 1957, Bruce-Chwatt 1985). Accordingly, information regarding the distribution of the principal vectors, *An. gambiae.s.s*, *An. arabiensis* and *An. funestus,* is generally based on these regional and continental extrapolations (Davidson and White 1972). However, since the spatial segregation and temporal heterogeneity of these vectors is governed by local climatic conditions particularly temperature and annual rainfall (Rogers et al. 2002), it is probable that the vectors in the Northern and wetter parts of the country are predominantly *An. funestus* and *An. gambiae s.s*. The remaining parts of the country are zones where *An. arabiensis* and *An. gambiae s.s* would dominate in various ratios depending on the time of the year, rainfall and temperature inter-annual variations (Chimumbwa 2003).

The first empirical evidence of the existence of malaria vectors implicated *An. gambiae s.l*, and *An. funestus s.l.* at the inception of malaria control activities in 1929 at Roan Mine in Luanshya (Watson 1953, Utzinger et al. 2001). Efforts to determine the distribution of these vectors were augmented by De Meillon (1937) and Adams (1940) who determined their flight ranges in the Copperbelt. Pielou (1947) confirmed the presence of *An. gambiae s.l.* and *An. funestus s.l.* and Paterson (1963) recorded the co-existence of *An. gambiae s.s*, *An. arabiensis* and *An. quadriannulatus* at Chirundu. Entomological studies in Chirundu confirmed the presence of endophilic and anthropophilic *An. gambiae* prior to experimental hut trials of DDT and HCH (Shelly 1973, Zahar 1985). Ten years later in the same area *An. gambiae s.s* had disappeared and was replaced by *An. arabiensis*, which was the predominant species in most parts of the country (Shelly 1973, Bransby-Williams 1979, Lindsay and Martens 1998). This suggested that insecticide utilization had changed the vector species composition, although extensive temporal data to support such a conclusion was not collected.

An. gambiae s.s and *Anopheles funestus* are usually more common in wetter areas, whilst *An. arabiensis* is better adapted to drier conditions (Lindsay and Martens 1998). Although earlier entomological surveys implicated *An. gambiae s.s* and *Anopheles funestus* as the predominant malaria vectors in Zambia (Bransby-

Williams 1979, Shelly 1973, Zahar 1985), recent studies conducted by Chimumbwa in 2002 at two spatially distinct sites (Kapululila village near Chirundu in Kafue district of Lusaka province, and Lukwesa village in Mwense district of Luapula province), indicated the co-existence of all three significant vectors of malaria, albeit at different densities. Lehmann et al. (2003) also examined the population structure of *An. gambiae* in southern Africa, and Weeto et al. (2004) collected *An. funestus* s.s. and *An. leesoni* Evans in eleven countries, including Zambia (Norris 2002). However, data on the distribution and speciation of Anopheline mosquitoes in Zambia is fragmentary and mostly collected from areas without vector control interventions (Lehmann et al. 2003, Weeto et al. 2004).

In order to direct future research and control efforts in Zambia, a study was conducted in 2003 prior to the introduction of the integrated vector management strategy. This demonstrated the sympatric existence of *An. gambiae s.s* and *An. arabiensis* in peri-urban Lusaka, where the former greatly out-numbered the latter. No *An. funestus* was identified in the study area (Chanda 2007). Other entomological studies in Macha, a low rainfall zone with hyper endemic malaria transmission, demonstrated the presence of *An. arabiensis, An. funestus* and *An. quadrianulatus* and established that transmission was maintained primarily by *An. arabiensis*, with *An. funestus* contributing secondarily in the apparent absence of *An. gambiae* s.s (Siachinji and Mulenga 2002, Siachinji et al. 2001, Kent et al. 2007). *Anopheles arabiensis* was substantially more anthropophagic in Macha than comparable populations of *An. arabiensis* from other parts of Africa (Kent et al. 2007), with significant temporal variation in densities observed between 2002 and 2005. Clearly there is great heterogeneity in vector species composition thus necessitating more expansive characterization of species in the country.

In sub-Saharan Africa populations of *An. gambiae s.s, An. arabiensis and An. funestus* are often sympatric, particularly within the 800 mm Isohyets of rainfall (Coetzee et al. 2000). *An. gambiae s.s* and *An. funestus* possess exceptional vectoral competence, attributable in part to their strong anthropophily and endophily, marked endophagy and relatively long lifespan (Besansky et al. 2004). Both species are characteristically amenable to control by IRS and ITNs (Protopopoff et al. 2007, Sharp et al. 2007). However, while malaria vector control efforts using IRS and

ITNs are being scaled up in Zambia, little effort has been made to determine the spatial and temporal impact of these interventions on species composition. To this effect, the malaria decision support system (MDSS) project (IVCC 2011) established eighteen monitoring sites in 2008 to assess the operational impact of IRS and ITNs on malaria transmission factors, including species composition and densities. Entomological data collected through the MDSS project will be used to augment existing data on malaria vectors in Zambia and to facilitate rational decision making for vector control.

1.11.5 Malaria Vector Species Infectivity Rates in Zambia

While *An. gambiae* s.s, *An. arabiensis* and *An. funestus*, differ in their vectorial capacity and population dynamics due to variations in their predilection for anthropophagy, dispersal and temporal activities, their distribution is also governed by annual precipitation (Rogers et al. 2002, Lindsay et al. 1998). As such, increases in their densities and infectivity usually coincides with the rainy season and produce spatial and seasonal diversity among sites (Keating et al. 2003). Mosquito density indicates the number of female *Anopheles* of a defined species caught sheltering in human dwellings or feeding on inhabitants (Bruce-Chwatt 1985). Vector infectivity is represented by the sporozoite rate i.e. the percentage incidence of sporozoite infection in the salivary glands of *Anopheles* mosquitoes.

There is limited information on vector infectivity for Zambia, and the available data shows great spatial and temporal heterogeneity in transmission potential of malaria vectors. Vector infectivity data on *An. gambiae s.l.* and *An. funestus* were collected in 1963 by researchers using hand-dissections at Chirundu and Livingstone in Southern province, Lusaka in Central province, Chipata in Eastern province and Ndola on the Copperbelt (Shelly 1973, Bransby-Williams 1979, Zahar 1985). Sporozoite rates ranged from 0% to 16% largely in areas devoid of vector control interventions. Sporozoite rates of *An. arabiensis* from Chipata and Lusaka were 0.4% for 981 female mosquitoes dissected, with a Human Blood Index (HBI) of 98% implying high anthropophagy.

Subsequent data on *Anopheles* sporozoite infectivity in Zambia were collected by the Malaria Research Laboratory between 1969 and 1970 (Zahar 1985). The

sporozoite rates for *An. gambiae* Giles *s.l.* and *An. funestus* from indoor and outdoor collections at Chirundu and Ndola ranged from 0% to 7.2%. These findings were higher than the sporozoite rates observed by Shelly (1973) or Bransby-William (1979) (Zahar 1985). *Anopheles arabiensis* was the only species identified from polytene chromosomes after *An. gambiae s.l.* collections from Southern, Eastern, Central provinces and the Copper belt. These fall within the low rainfall zone of the country (Shelly 1973, Bransby-Williams 1979, Zahar 1985).

Sporozoite rates determined in 2000 for *An. arabiensis* were 5.6% in a low rainfall zone at Kapululila near Chirundu and 5.9% and 4.4% for *An. gambiae s.s.* and *An. funestus* respectively at Lukwesa, a high rain fall zone of Luapula province (Chimumbwa 2003). *Anopheles arabiensis* proliferates even in arid conditions, thus this species has high transmission potential in such areas. No sporozoites were detected in *An. gambiae s.s.* and *An. funestus* samples at Kapululila or in *An. arabiensis* at Lukwesa. Entomological studies were conducted in 2002 to determine the major malaria vectors at Macha. The sporozoite rate was 4.23% in *An. arabiensis* (Siachinji and Mulenga 2002). Kent et al (2007) further determined sporozoite rates for *An. arabiensis* from Macha in the Southern province. Average sporozoite rates ranged from 0% in 2005 during the period of drought to 1.6% at Chidakwa to 18.2% at Lupata in 2006. Although *An. funestus* s.s is a major vector it is sensitive to drought and changing environmental conditions (Mouchet et al. 1996). The HBI for *An arabiensis* was 92% indicating that transmission was driven solely by *An. arabiensis* in the 2005 to 2006 transmission season. During the drought period, only one sporozoite-positive *An. arabiensis* and one sporozoite-positive *An. funestus* s.s was collected between November 2004 and May 2005, implying that indeed climatical factors have an effect on the distribution and abundance of malaria vectors. These findings indicate that there is a great diversity in transmission potential of the major vectors of malaria in Zambia.

1.11.6 Insecticide Resistance in Zambia

Zambia was one of the countries to augment their malaria vector control efforts with IRS from 1950s, in line with the WHO call for the global eradication of malaria through the use of DDT (WHO 1957, Utzinger et al. 2002). During this time, there was limited entomological monitoring including surveillance for insecticide

resistance. This effort ceased in the early 1980s largely due to economic constraints (MoH 2000). The revival of malaria vector control efforts in Zambia, in the wake of establishment of the Roll Back Malaria partnership (MoH 2000), has stimulated unprecedented local and international support for implementation and monitoring of malaria interventions. The use of ITNs and IRS with DDT and pyrethroids, has been scaled-up with a concomitant reduction in malaria-related morbidity and mortality (Chanda et al. 2008).

Sporadic data dating back to 1999 on the resistance status of vectors prior to implementation of interventions during the scaling-up has been collated in Zambia (NMCC, unpublished data). This showed full susceptibility of all the three vectors to all candidate public health insecticides. However, the extensive exposure of vector species to insecticides through community-based mass distribution of ITNs and IRS with pyrethroids and DDT is a challenge that is likely to select for resistance. Equally, the cultivation of crops that require regular pesticide application such as cotton, coffee, sugar cane, bananas and vegetables in Zambia, will undoubtedly increase insecticide selection pressure on the malaria vectors. To ensure that insecticide choice for malaria vector control is effective and evidence-based, routine monitoring of potential resistance mechanisms within target populations needs to be undertaken to preserve and prolong the utility of current vector control tools in the country.

In an effort to optimally quantify and manage insecticide resistance in operational settings, the NMCP in Zambia has established eighteen sentinel sites through the malaria decision support system to facilitate resistance surveillance and monitoring (IVCC 2011).

1.12 Background and Aims of this Present Study

1.12.1 Background of the Study

Studies on the comparative operational impact of IRS and ITNs on malaria transmission (Neville et al. 1996), have demonstrated that both interventions are effective in a large number of epidemiological settings (Lengeler and Sharp 2003). Choosing between the two is largely a matter of operational feasibility and

availability of local resources rather than one of malaria epidemiology or cost effectiveness (WHO 2005, Pardo et al. 2006). However, most malaria control programmes apply insecticides for disease control on the basis of incomplete and often anecdotal data and/or general guidelines. For example data about insecticide resistance in the vector population are either obtained in an ad hoc manner or inferred from the apparent failure of vector control (Hemingway et al. 2006).

In order to improve decision support tools for vector control, it is critical to optimize impact assessment of entomological interventions. The malaria decision support system developed through the Innovative Vector Control Consortium (IVCC) has been designed to achieve this goal. A more directed and efficient monitoring of entomological and epidemiological parameters related to transmission will inform effective vector control through focused application of interventions (Hemingway et al. 2006).

While vector control interventions are being deployed in line with the WHO-led IVM strategy (Beier et al. 2008, Chanda et al. 2008), their implementation has been anecdotal and mostly based on assumptions and expert opinions. Monitoring and evaluation has been fragmented, irregular, uncoordinated and lacked a spatial and temporal framework. If transmission determining parameters are to be harnessed effectively for decision-making to objectively plan, implement, monitor and evaluate viable options for malaria vector control (Smith et al. 2005), they must be properly monitored.

The use of both IRS and ITNs in Zambia provides an opportunity to compare and optimize the assessment of their impact on malaria transmission determinants in meso-to hypo-endemic operational settings. In this regard, shifts in the vector resistance status, species abundance, sporozoite rates and parasite prevalence including deaths and case fatality rates that have followed in the wake of consistent deployment of these interventions should be monitored to generate pragmatic data to inform policy and optimise interventions in the country.

1.12.2 Aims and Objectives of the Thesis

Rationale: Vector control is critical in reducing malaria transmission to humans and the related morbidity and mortality. Evidence-based deployment and optimal assessment of transmission-reducing tools allow for viable policy formulation for control.

To implement effective vector-based intervention strategies, increased knowledge on the interactions of epidemiological and entomological malaria transmission determinants is needed in the assessment of impact of interventions. To address this, the goal of this study was to optimize data collection around programmatic impact assessment of IRS and ITNs on malaria transmission and vector bionomics in operational settings by:

1) Demonstrating that impact assessment of IRS and ITNs or both combined on malaria transmission can be optimized by using population based *P. falciparum* parasite prevalence surveys and routine surveillance data within operational areas of low transmission intensity.

2) Validating the premise that extensive implementation of IRS and ITNs result in a significant reduction in species abundance and infectivity of indoor resting malaria vectors in operational settings.

3) Demonstrating the impact of IRS and ITNs on the phenotypic and genotypic levels of insecticide resistance status in vectors and inherent mechanisms in order to inform policy and suggest insecticide resistance management strategies.

4) Showing the significance of a malaria decision support system in optimizing impact assessment of IRS and ITNs and in facilitating malaria vector control policy formulation.

CHAPTER TWO

General Materials and Methods

2.1 Study Sites and Population

Zambia is situated in the Southern African region between 8^{o} and 18^{o} degrees south latitude and between 20^{o} and 35^{o} degrees east longitude with an area of 752,614 sq km, out of which 740,724 sq km are land and 11,890 sq km water. The country is landlocked, sharing borders with Mozambique in the southeast, Zimbabwe and Botswana in the south, Namibia in the southwest, Angola in the west, Democratic Republic of Congo (DRC) in the north, and Tanzania in the northeast and Malawi in the east (Figure 2.1). The population of Zambia is approximately 12 million, 45% of whom are below the age of fifteen, based on a growth rate of 2.11%, from the last complete census (CSO 2000). The country is divided into nine provinces and 72 administrative districts run by local authorities. The districts are the basic planning levels for health service delivery.

Topographically, Zambia consists largely of a highland plateau with elevations ranging from 915 to 1,520 metres above sea level. The country's vegetation is of the savanna woodland type in high rainfall regions and tropical grassland type in low rainfall regions (Fanshawe 1971, Storrs 1995). The most extensive savannah woodlands are Miombo woodlands, found more abundantly in the north and north-west than the south, that covers about 42% of the country (ECZ 2000).

There are three distinct seasons: a cool and dry season from April to August, a hot and dry season from August to November and a warm and rainy season from November to April. The average temperatures range from 16^{o} to 27ºC in the cool dry season and from 27^{o} to 38ºC in the rainy and hot season, and vary as a function of altitude. Rainfall decreases from north to south with an average annual rainfall from 600 mm in the south to 1400 mm in the north per year, peaking between November and March.

2.1.1. Malaria Vector Control Interventions

In response to the high burden of malaria in Zambia, a robust malaria control programme was established including vector control through incremental deployment of indoor residual spraying (IRS) in urban and peri-urban areas and ITNs particularly in rural areas (MoH 2006). Indoor Residual Spraying was

implemented incrementally from 5 districts in 2003 to 36 districts in 2008 with scale up to 54 and 72 districts planned for in 2010 and 2012 respectively. During the 2009 IRS campaign, over 1.2 million households were sprayed, protecting over 4.5 million people (MoH 2009). Indoor Residual Spraying, with the goal of covering at least 85% of eligible households in targeted areas, is deployed through annual campaigns using pyrethroids (deltamethrin and alpha-cypermethrin, (Bayer); and lambda cyhalothrin, (Syngenta) and DDT (Avima). Indoor Residual Spraying is carried out prior to peak malaria transmission that coincides with the rainy season from November to April (MoH 2010). Spray operations are in line with country specific guidelines, adapted from the WHO guidelines (WHO 2006, MoH 2010). Computerised spray management systems were used to continually monitor the progress and performance of spray operations (Booman et al. 2003).

Insecticide Treated Net distribution strives towards attaining a goal of 100% coverage and at least 85% utilization rates in all eligible areas. Insecticide Treated Net coverage has been increasing since 2000 via several distribution mechanisms, including ant-natal and child clinics, commercial, school health programmes and recently mass distributions of 3 LLINs per household since 2005. Over 7 million ITNs have been distributed with 75% of households possessing at least one net (Keating et al. 2009). The distribution of ITNs is strictly in accordance with the country specific guidelines adapted from WHO with a two component monitoring system (1) compilation of information on number of ITNs distributed and (2) tracking ITN coverage/ownership and utilization rates by households (MoH 2008).

Vector control programmes are coordinated and managed by the Ministry of Health through the National Malaria Control Centre (NMCC). Implementation of interventions at district level is done by the District Health Management Teams (DHMT) in collaboration with community members.

A successful control programme is a combination of effective vector control and case management. ICT Malaria Test® (R and R marketing, Cape town, South Africa) and SD Bioline Malaria Ag pf® (Standard Diagnostics Inc., Suwon city, South Korea) rapid diagnostic tests (RDTs) were introduced country-wide in 2006 to support microscopy for diagnosis of malaria cases. First line treatment is currently

with Coartem® (artemether/lumefantrine, AL), an artemisinin-based combination therapy (ACT) for uncomplicated malaria, and quinine is the second line treatment policy for complicated malaria (Sipilanyambe et al. 2008, Barnes et al. 2009, Keating et al. 2009). Intermittent preventive treatment (IPT) (a curative dose of an antimalarial drug given at fixed times to high-risk groups, such as pregnant women and infants, regardless of infection) while three doses of sulphadoxine-pyrimethamine (SP) are given to expectant mothers, at one month intervals at prenatal visits in the last six months of pregnancy. This is further augmented with interactive information, education and communication (IEC) and behavioural change and communication (BCC) strategies to enhance utilization of interventions.

Figure 2.1: Map of Zambia showing the location of the neighbouring countries in Southern Africa. (Source: http//www.un.org/Depts/Cartographic/map/profile/Zambia.pdf).

2.1.2 Sentinel Sites

During this project, a system of 18 sentinel sites, distributed amongst nine districts within a radius of 350 Km from Lusaka (Figure 2.2), was developed for monitoring and evaluation. Data collection included an annual household survey that included parasite prevalence, insecticide resistance, mosquito abundance and infectivity. The

selection of sentinel sites was based on the following criteria; cost; pre-existence of data; current and previous vector control interventions, and environment. The sentinel sites were located in an area characterized by low seasonal transmission of malaria with a wide coverage of vector control interventions.

Of the nineteen monitoring sentinel sites, IRS was carried out in 5 sites, Kabulongo and Mufweshya in Chongwe district, Kafue estates in Kafue district and Chimoto and Mukobeko in Mumbwa and Kabwe districts respectively. Vector control in the remaining fourteen sentinel sites, Chunga, Myooye and Chimoto in Mumbwa district and Chibombo, Mulungushi and Chisamba in Chibombo district, Chipepo in Kapiri mposhi district, Manueli and Nyamankalo in Luangwa district, Rufunsa in Chongwe district, Chikankata, Munenga and Mwanachingwala in Mazabuka district, Chiawa in Kafue district and Chobana in Monze district is by ITNs. Both Perma Net®, Verstargaard Frandsen and Olyset®, Sumitomo, were distributed in these areas (MoH 2008).

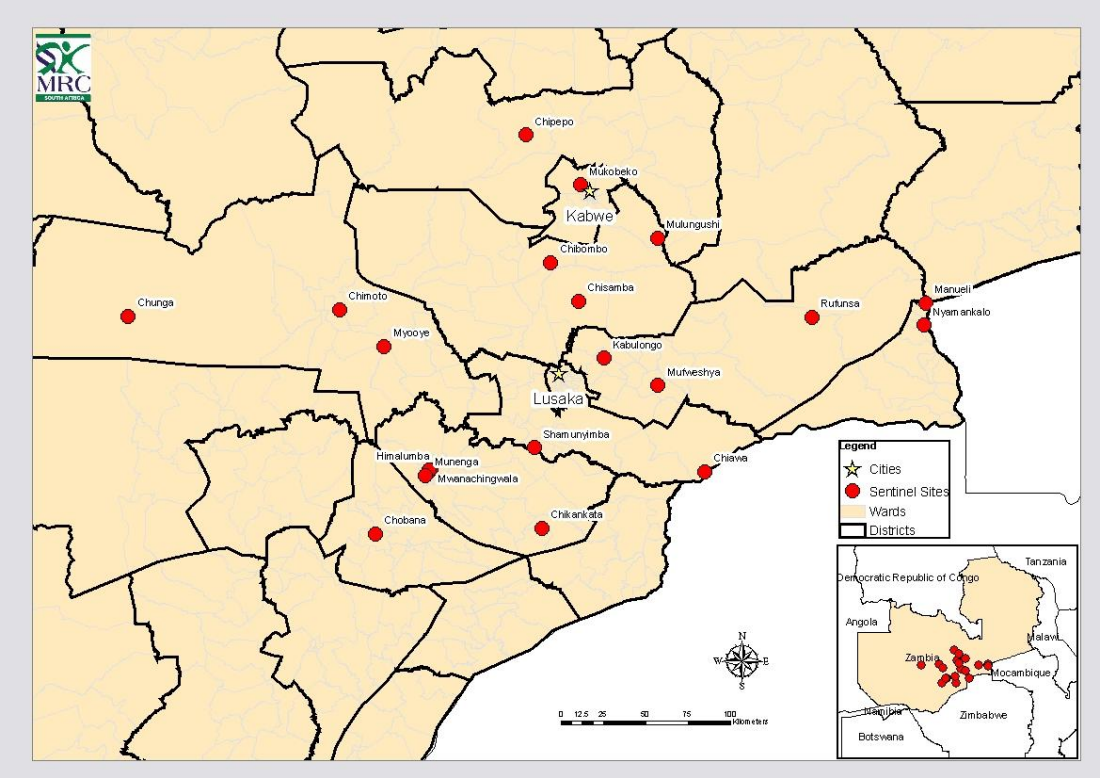


Figure 2.2: .Map of Zambia showing the location and distribution of the 19 malaria decision support system monitoring sentinel sites.

2.2. Entomological Monitoring

2.2.1 Mosquito Collections for Resistance

2.2.1.1 Mosquito Larval Collections

Following an assessment of potential breeding sites in each study locality, larvae were collected by the dipping method using 200 ml capacity ladles and transferred into white enamel bowls for sorting. Larvae were collected irrespective of stage. After removing culicine larvae and most of the other aquatic fauna including predators, anopheline larvae were transported back to the laboratory and reared to adults for insecticide resistance testing.

2.2.1.2 Adult Catches by Aspiration

Live indoor resting blood fed adult female *An. gambiae s.l* and *An. funestus s.l.* were collected from resting surfaces inside homes using an aspirator as described by Service (1976) during the period April 2008 to May 2010, from 17 localities in Zambia, 11 of which were sentinel sites (Figure. 2.2). Collections were conducted, with home owner's informed consent, early in the morning between 06.00 and 08.00 hours. Although specific houses varied, collections were made from at least ten houses at each study site during each field visit in order to provide a representative sample. To avoid genetic bias, mosquitoes were collected from intervention and non intervention homes (Service 1977, Service 1976, W.H.O 1975). Collected mosquitoes were transported to the laboratory, transferred to individual oviposition tubes, and females allowed to lay eggs. Larvae were reared separately through to 1-3-day-old F1 adults under controlled insectary conditions of 26 ± 2ºC and 70-80% Relative Humidity, photoperiod 12:12 L: D hours as described by WHO (1975).

2.2.1.3 Mosquito Collections for Species Abundance and Infectivity

Following WHO (1975) procedures, window exit traps were used to monitor adult *Anopheles* mosquitoes' abundance and infectivity from sentinel sites. With homeowners consent, exit window traps were installed at six houses at each of the eighteen sentinel sites in April 2008. The nineteenth sentinel site was not used for exit window trap collections as it was located in a game park area with all houses made from concrete blocks and metallic roofing materials. Mosquito collections

were made daily by home owners trained to empty the contents of the window trap, in the morning between 07.00-08.00 hours into a pre-labeled specimen jars containing isopropanol. Checklists were completed specifying nights for which traps were not operating. Jars were collected and replaced at four week intervals.

2.2.2. Mosquito Species Identification

2.2.2.1 Morphological Identifications

The collected mosquitoes were segregated into *Anophilinae* and *Culicine* and enumerated. The female *Anopheles* mosquitoes were identified morphologically as *An. gambiae* complex or *An. funestus* group using keys (Gillies and De Meillon 1968, Gillies and Coetzee 1987) and individually stored in 100% isopropanol or silica gel in Eppendorf tubes for subsequent analysis.

2.2.2.2 Polymerase Chain Reaction (PCR) Identifications

Sibling species within the *An. gambiae* and *An. funestus* complexes were identified using the cocktail polymerase chain reaction (PCR)-single strand conformation polymorphism (SSCP) procedure of Koekemoer et al. (2002) and the ribosomal DNA-polymerase chain reaction (PCR) technique of Scott et al. (1993) respectively. The sibling species within the *An. nili* group and *An. funestus*-like species were identified according to the multiplex PCR technique described by Kengne et al. (2003) and the protocol described by Spillings et al. (2009) respectively.

2.2.2.3 *Anopheles gambiae* Complex

One leg per mosquito was removed and used for species identification according to the polymerase chain reaction (PCR) method described by Scott et al. (1993) and standardized according to Van Rensburg et al. (1996). Primers AR, GA, ME and QD amplify products diagnostic for *An. arabiensis* (315 bp), *An. gambiae* (390 bp), *An. merus* (466 bp) and *An. quadrianulatus* (153 bp) respectively. The leg was put into a PCR reaction mixture containing: 1.25 µl 10X PCR buffer, 125 µM of each of the four nucleotides; 1mM $MgCl_2$; 0.3 µM of the universal primer UN, GA, AR and ME primers and 0.15 µM QD primer; 0.5 units taq DNA polymerase. Species standards were drawn from colony material and run concurrently with all assays. PCR reaction conditions were run at 94^{o}C for 30 sec, 50^{o}C for 30 sec and 72^{o}C for 30 sec for 30

cycles with a final extension step at 72^{o}C for 10 min. The amplified fragments were analysed using a 2% agarose gel stained with ethidium bromide and visualized under UV light.

Primer name	Species name	Primer sequences (5' to 3')	Band size
UN	-	GTG TGC CCC TTC CTC GAT GT	-
GA	*An. gambiae s.s*	CTG GTT TGG TCG GCA CGT TT	390
ME	*An. merus*	TGA CCA ACC CAG TCC CTT GA	466
AR	*An. arabiensis*	AAG TGT CCT TCT CGA TCC TA	315
QD	*An. quadrianulatus*	CAG ACC AAGATGGTT AGT AT	153

Table 2.1: Primer sequences of species-diagnostic *An. gambiae* complex. UN = Universal. (Reproduced from Scott et al., 1987).

2.2.2.4 Molecular Forms (M/S) of *Anopheles gambiae s.s*

Determination of the molecular form (M/S) of each specimen was done by the diagnostic PCR-based assay described by Favia et al. (2001). One leg of female *An. gambiae s.s* specimens was put in a 50µl reaction mixture prepared by using 1.25 units of Taq DNA polymerase, 0.2 mM each dNTP, 1µM primer, and 1 µl of DNA resuspended in 100 ml of TE buffer. The reaction was cycled 40 times through the following scheme: 45 sec at 94°C, 45 sec at 50°C, and 1.2 min at 720C. The amplification products were separated electrophoretically on a 1.4% agarose gel and visualized with UV light. For the tRNA gene amplification the following primers were used:

Primer name	Sequence (5'to 3')
T1	ATCCATAGGTCGCTGGTTC
T2	CGATAGCTCAGTTGGTAGAG
T3	TCGTGGCCGAGTGGTTAA
T4	TAGCTCAGTTGGTAGAGC

Table 2.2: Primer sequences of diagnostic molecular forms (M/S) of *An. gambiae s.s* (Reproduced from Flavia et al, 1994).

2.2.2.5 *Anopheles funestus* Group

Wild-caught specimens and those from families reared from wild families belonging to the *An. funestus* complex were identified to sibling species using the cocktail polymerase chain reaction (PCR) - single strand conformation polymorphism (SSCP) procedures of Koekemoer et al. (2002) and Spillings et al, (2009). Primers FUN, VAN, RIV, PAR, LEES and FUNL amplify products diagnostic for *An.*

funestus s.s (505 bp), *An. vaneedeni* (587 bp), *An. rivulorum* (411 bp), *An. parensis* (252 bp), *An. leesoni* (146 bp) and *An. funestus*-like (390 bp) respectively in conjunction with the universal primer UN. A mosquito leg was put into the PCR reaction mixture containing: 12.5-µL reactions contained the following: 1.25 µL 10 X reaction buffer (500 mM KCl, 100 mM Tris-HCl pH 8.3), 1.5 mM $MgCl_2$, 3.3 pmol/primer of each primer, 200 µM of each dNTP, and 0.5 units thermo stable taq DNA polymerase overlaid by a drop of mineral oil. PCR cycles were as follow: One cycle at 94°C for 2 minutes followed by 30 cycles at 94°C for 30 seconds, 45°C for 30 seconds, and 72°C for 40 seconds. An additional auto extension of 72°C for 5 minutes was included for one cycle at the end. PCR products were electrophoresed on 2.5% agarose gel stained with ethidium bromide. Primer names, sequences as well as Tm temperatures are provided below:

Primer name	Species name	Primer sequences	Band sizes	Tm (^oC)
UV	-	TGT GAA CTG CAG GAC ACA T	-	-
FUN	*An. funestus s.s*	GCA TCG ATG GGT TAA TCA TG	505	52.4
VAN	*An. vaneedeni*	TGT CGA CTT GGT AGC CGA AC	587	58
RIV	*An. rivulorum*	CAA GCC GTT CGA CCC TGA TT	411	58.8
PAR	*An. parensis*	TGC GGT CCC AAG CTA GGT TC	252	60.5
LEES	*An. leesoni*	TAC ACG GGC GCC ATG TAG TT	146	60.2
FUNL	*An. funestus*-like	GTT TTC AAT TGA ATT CAC CAT T	390	-

Table 2.3: Primer sequences of species-diagnostic *An. funestus* complex with expected band sizes. UV = Universal (Source: Koekemoer et al., 2002 and Spillings et al., 2009).

2.2.2.6 *Anopheles nili* Group

One leg of each mosquito was removed and used for species identification according to the multiplex polymerase chain reaction method for *An. nili* group described by Kengne et al. (2003). The size of the diagnostic band is 188 bp for the typical (T) *An. nili*, 357 bp for *An. ovengensis*, 408 bp for *An. carnevalei* and 329 bp for *An. somalicus* respectively in conjunction with the universal primer UN. PCR reaction mixture consisted of 1.5 mM $MgCl_2$, 200µM each of dNTP, 2.5 µL 10X Taq buffer, 0.625 U Taq polymerase and 10ng of template DNA in 25uL final reaction volume. The amount of each primer used in the PCR assay was 40 pmol for ANU, and 10 pmol each for ANT, ANO, ANC and ANS. PCR conditions included an initial denaturation step at 94^{o}C for 5 min, followed by 30 cycles of 30 sec at 94^{o}C, 30 sec at 63^{o}C and 1 min at 72^{o}C. The amplified fragments were separated by

electrophoresis on 2% agarose gel stained with ethidium bromoide and visualized under UV light.

Primer name	Species name	Primer sequence (5' to 3')	Band size	Tm (^{o}C)
ANU	-	GAT GCA CAC ATT CTT GAG TGC C	_	52
ANO	*An. ovengensis*	AGC ACGGTC ACC TAC GGT TCT CC	357	56
ANC	*An. carnevalei*	CTG GTG GGG TTC TTC TCT TCT CG	329	55
ANT	*An. nili s.s*	TGG CTG CTT CTC GTG GCG CG	188	56
ANS	*An. somalicus*	ATG CAC CAG GGG GTT TGG GCC	329	56

Table 2.4: Primer sequences of species-diagnostic *An. nili* complex with expected band sizes and Tm temperatures. ANU = Universal. (Source: Kengne et al., 2003).

2.2.3 WHO Susceptibility Tests

Insecticide susceptibility assays were carried out on a random sample of 1-3-day-old, sugar fed F1 and F0 adult male and female mosquitoes from each family following the procedure described by the World Health Organization (WHO 1998). The use of sugar-fed, 1-3-day-old adults allowed standardization of age, physiological state, and testing conditions for all assays, in contrast to the mixed age, wild-caught mosquitoes, where age and pre exposure to insecticides would have influenced the assay results. However due to the scarcity of the malaria vectors in some localities, the numbers were supplemented with F0 adults reared from larvae. F0 from larvae have the same non-exposure and physiological state, allowing standardization. Insecticides tested included 1) the pyrethroids; lambda-cyhalothrin (0.05%), deltamethrin (0.05%), and permethrin (0.75%), 2) the carbamates; bendiocarb (0.01%) and propoxur (0.01%), 3) the organophosphate malathion (5%) and 4) DDT (4%). Between 20 and 25 adult mosquitoes were exposed to insecticide-impregnated filter paper or control papers impregnated with the insecticide carrier oil for 1h and then transferred to clean holding tubes and supplied with 10% sugar solution for 23h, after which percentage mortality was determined.

Random samples of insecticide treated papers used to test F0 mosquitoes reared from larvae or F1 progeny were tested for efficacy on susceptible laboratory colony of *An. arabiensis* from Macha Malaria Institute in Choma district of Zambia. Following experiments mosquitoes were preserved in individual labelled Eppendorf tubes with silica and stored at room temperature. When control mortality was

between 5 and 20% it was corrected by applying the Abbots formula. If control mortality was >20%, the data was discarded and the test was repeated. All test kits and insecticide-impregnated papers were supplied by the WHO.

The Chi squire test was used to determine whether there was significant difference in resistance levels between the present findings and the previous data.

- *Chi square:* $X^2 = \sum(O - E)^2/E$ = Sum of Number of [(observed number – expected number)^2] ÷ expected number.
  Where
  X^2= Sum of total number of $(O - E)^2/E$ computations
  O = Observed number
  E = Expected number

2.2.4 DNA Extraction Assay

DNA was extracted from single mosquitoes by using the Livak method according to the protocol of Collins et al, (1987). Only the *Anopheles* heads and thoraces were processed for infectivity to confirm that sporozoites are detected and not other parasite life stages that would be present in the abdominal cavity, to reduce the error and effects of estimating malaria infection rates, as recommended by Beier and Koros (1991). Each mosquito was homogenized in 200µl grinding buffer (0.08M $NaCl_2$; 0.16M sucrose; 0.06M EDTA; 0.5% SDS and 0.1M Tris-Cl pH 8.6), and then incubated at 70^oC for 30 minutes following which 28 µl of 8M potassium acetate was added. Protein precipitation was then achieved following incubation on ice for 30 min. The precipitated protein and other debris was removed by centrifugation at 16,000 rpm for 10 min. The supernatant from each sample was transferred to a new tube and mixed with 400 µl absolute ethanol. The DNA from each sample was then precipitated by centrifugation at 16,000 rpm for 30 min. Salts were washed from each remaining DNA pellets using 70% ethanol following which the pellets were allowed to air dry. Each pellet was then resuspended in 200µl 1 X TE buffer.

2.2.5 Knockdown Resistance (kdr) Detection

To detect the west-type kdr, the diagnostic PCR described by Martinez-Torres et al. (1998) that detects a single amino acid substitution in region II of the par-type sodium gate channel was used to distinguish between ‘resistant’ and ‘susceptible’

kdr alleles in resistant homozygotes and heterozygotes from the field populations of *An. gambiae s.s*. Genomic DNA was added to 25µl of PCR mixture containing : 2.5 µl of 10 X buffer (100 mM Tris-HCl, ph 8.3, 500mM KCl), 1 mM $MgCl_2$, 2.5 µl of a 2.5 mM of each dNTP, 0.3 mM each of primers AgD1, AgD2, AgD3 and AgD4 and 1 unit Thermostable taq DNA polymerase. Volume was made up to 25 µl by adding ddH_2O. PCR reaction conditions were standardized at 94^oC for 1 min, 48^oC for 1 min and 72^oC for 1.5 min for 35 cycles with a final extension step at 72^oC for 10 min. The amplified fragments were analyzed using a 2.5% agarose gel stained with ethidium bromide and visualized under UV light (Table 2.4). Primers AgD1 and AgD2 that frank the region containing the kdr mutation amplify a 293 bp product from common genomic DNA fragment. Primers AgD3 and AgD4, nested within this region, were allele specific. Primer AgD3 binds only to the resistant kdr allele and, when paired with AgD1, will amplify a 195 bp fragment if this allele is present in the individual. AgD4 binds only to susceptible allele and will pair with AgD2 to produce a 137 bp band if the susceptible allele is present (Martinez-Torres et al. 1998). Mosquitoes heterozygous at this locus show all three PCR products.

The diagnostic PCR described by Ranson et al. (2000) was used to detect the east-type kdr mutation by substituting primer AgD3 for AgD5 (Ranson et al. 2000). 0.75% of the total genomic DNA extracted from a single mosquito was used as a template in a 15 ml PCR reaction containing 2 mM $MgCl_2$, 0.2 mM dNTP, 0.5 mM of primers AgD4 and AgD3, 0.3 mM of primers AgD1 and AgD2 and one unit of Taq DNA polymerase in 20 mM Tris–HCl and 50 mM KCl. The PCR conditions were 94 °C for 5 min and then thirty cycles of 94 °C for 25 s, 55 °C for 20 s and 72 °C for 8 s with a final 10 min extension at 72 °C (Table 2.5).

Primer name	Sequence (5’ to 3’)
AgD1	ATA GAT TCC CCC GAC CAT G
AgD2	AGA CAA GGA TGA TGA AAC C
AgD3	AGA CAA GGA TGA TGA ACC
AgD4	AAT TTG CAT TAC TTA CGA CA
AgD5	CTG TAG TGA TAG GAA ATT TA

Table 2.5: Primer sequence for the kdr diagnostic PCR. (Reproduced from Martinez-Torres et al., 1998 and Ranson et al., 2000).

2.2.6 Vector Abundance and Infectivity

Numbers of mosquitoes per trap per night were calculated for each vector species based on day of capture of the specimen. The species specific sporozoite prevalence; the number of infected mosquitoes per trap per night (transmission index) by species was calculated and pattern of pyrethroid knock down resistance (kdr) was determined.

Traditionally, sporozoite rates have been determined by manually examining salivary glands for sporozoites, by ELISA with the circumsporozoite protein (CSP) serving as the target antigen (Burkot et al. 1984, Wirtz et al. 1987) and by PCR. Although ELISA is the most common method cited in the literature, it requires that the specimens be screened fresh in the field or maintained by cold chain, and complications due to false positives have been reported (Beier et al. 1990, Povoa et al. 2000, Somboon et al. 1993). *An. gambiae s.s, An. arabiensis* and *An. funestus s.s* collected from exit window traps in this study were sorted into species, and tested for the presence of *P. falciparum* circumsporozoite protein using the TaqMan assay protocol described by Bass et al, (2008).

2.2.6.1 *Plasmodium falciparum* Sporozoite Detection

Plasmodium falciparum sporozoite rates were determined by using the TaqMan assay described by Bass et al., (2008). Assay conditions: PCR reactions (20 µl) contained 1 µl of genomic DNA, 10 µl of SensiMix DNA kit (Quantace), 800 nM of each primer (PlasF 5'-GCTTAGTTACGATTAATAGGAGTAGCTTG-3' and PlasR 5'-GAAAATCTAAGAATTTCACCTCTGACA-3') and 300 nM of probe PlasF (Falcip+ 6FAM-TCTGAATACGAATGTC) and 200 nM of probe OVM+ (OVM+ VIC-CTGAATACAAATGCC). Assay PCR cycle conditions: PCR reactions were run on a Rotor-Gene 6000™ (Corbett Research) using the temperature cycling conditions of: 10 minutes at 95°C followed by 40 cycles of 95°C for 10 seconds and 60°C for 45 seconds. The increase in VIC and FAM fluorescence was measured at the end of each cycle by acquiring each cycle on the yellow (530 nm excitation and 555 nm emission) and green channel (470 nm excitation and 510 emission) of the Rotor-Gene respectively.

The malaria transmission determining parameters; sporozoite rates, number of

mosquitoes per trap per 100 nights, transmission index and the relative transmission index, percentage proportion of species and their estimated numbers were computed using the following formulae;

- *Sporozoite rate* = The number of *Anopheles* infected with sporozoites ÷ The total number of *Anopheles* tested for sporozoites.
- *Number of mosquitoes per trap per 100 nights* = [(Total number of *Anopheles* mosquitoes collected ÷ Total number of collection nights)] ÷ Total number of exit traps x 100.
- *Transmission index* = Number of mosquitoes per trap per night x sporozoite rate.
- *Relative transmission index* = Transmission index ÷ Transmission index at baseline.
- *An. gambiae s.s proportion (%)* = (Total number of *Anopheles gambiae s.s* ÷ Total number of *Anopheles gambiae s.l*) x 100.
- *An. funestus s.s* proportion (%) = (Total number of *Anopheles funestus* s.s ÷ Total number of *Anophele funestus s.l*) x 100.
- *Estimated number of An. gambiae* s.s = proportion of *Anopheles gambiae s.s* caught
- *Estimated number of An. arabiensis* = proportion of *Anopheles arabiensis* caught
- *Estimated number of An. funestus s.s* = proportion of *Anopheles funestus s.s* caught

2.3 Epidemiological monitoring

2.3.1 Household Survey on Prevalence of Infection

Household surveys were conducted at the end of the malaria transmission season in April/May for three consecutive years: 2008, 2009 and 2010 using a survey questionnaire based on the model developed by the measure DHS+ programme and adopted and recommended by the RBM MERG task force on household surveys (W.H.O 2003). Malaria specific issues covered in the survey include an IRS and ITN survey. Inclusion of households in the survey, pre-selected by applying a simple sampling frame and geo-referencing, was used based on informed consent. All households at each sentinel site were enumerated and their coordinates were taken using Dell Axim X50 (Dell, Round Rock, TX) personal digital assistants (PDAs) equipped with Compact Flash (Next Warehouse.com, Tustin, CA) global positioning system (GPS) devices. Sentinel sites were considered as the primary sampling unit. Logistic regression, allowing for complex survey designs, was performed to estimate

the mean effect of the vector control intervention on prevalence compared to baseline prevalence of infection across years.

Information about the impending survey was given and relevant permissions sought and houses were marked according to the sampling frame and coordinates taken. Household surveys were conducted at the end of the malaria transmission season in April/May in 2008, 2009 and 2010. Households were selected from strata formed by dividing sentinel sites into quadrants from which 140 children aged 1 to <15 were randomly selected, to ensure the greatest geographical spread within the site. Written informed consent was sought from the responsible person at each selected household. Consenting householders were asked about attitudes towards IRS and ITNs, whether their house had been sprayed in the past year or whether they possess an ITN. The sentinel site specific sample size was calculated to provide evidence at the 5% significance level of an absolute reduction in *P. falciparum* prevalence of 20% (Korenromp et al. 2004). Prevalence and 95% confidence intervals (CI) for each sentinel site were estimated taking account of clustering by sentinel site using the statistical software package STATA (StataCorp LP. Stata Statistical Software: Release 10. College Station, TX, USA.).

2.3.2 Malaria Parasite Prevalence Survey

The design of this annual survey was to monitor the impact of the malaria vector control interventions at each of the nineteen sentinel sites. Children were tested for *Plasmodium falciparum* infection using ICT™ malaria combo rapid diagnostic tests (R&R, Cape Town, South Africa). The sensitivity of this kit has been assessed in the laboratory and field tested against other RDTs and blood microscopy (Craig et al. 2002). Children testing positive for *P. falciparum were* offered treatment with Coartem® (artemether-lumefantrine) according to the NMCP guidelines. Any complicated malaria case was referred to the nearest health centre.

Parasite detection was restricted to children under fifteen years because prevalence surveys in non-immune persons such as children give a good indication of the reservoir of infection in a population, and thus of transmission potential (Kleinschmidt et al. 2006). Additionally, morbidity and mortality due to malaria has

been shown to be high in this age group particularly children under fives of age in addition to pregnant mothers (Gamble et al, 2006; Snow et al, 1999; WHO, 2003).

Prevalence was calculated annually for each sentinel site and 95% confidence intervals were calculated using variance estimates that took account of clustering by sentinel site using the Rao and Scott correction (Rao and Scott 1981). With the assumption that there would be on average two children between 1 and < 15 years of age in each household, it was decided to set a target of approximately 40 homes per sentinel site for each survey round to compensate for the fact that school children may not be available during visits, and to allow for a design effect that would arise from within–household correction of responses at each sentinel site.

2.3.3 Routine Case Surveillance

Case data on children less than 5 years old was obtained from the Zambian national Health Management Information System (HMIS).

2.3.3.1 Case Definition

Malaria is diagnosed using direct microscopy in hospitals or clinics and by use of RDTs in rural health facilities and at community level. The latter are implemented under the Home Management of Malaria program where microscopy services are absent. Clinical diagnosis is used to define cases which have not been diagnosed by either microscopy or RDTs. Only confirmed malaria cases by either direct microscopy or RDT (ICT Malaria Test® R and R marketing, Cape Town, South Africa) were included in this study.

2.3.3.2 Study design

Routine surveillance data from the HMIS of the Ministry of Health were analyzed retrospectively. Data on malaria trends in Zambia is comprehensive with a complete HMIS with over 95% district monthly reporting rates.

Comparative information was obtained from two published nationally representative cross-sectional population-based Malaria Indicator household surveys (MIS) conducted in 2006 and 2008 (MoH 2006, MoH 2008). The Demographic Health Survey (DHS) also reported data on malaria morbidity and mortality and coverage

of interventions in 2007 (CSO 2007). A desk-based analysis assessed the programmatic management and the epidemiological impact of IRS and LLINs in children below the age of five years, using malaria related morbidity and mortality data from from HMIS and household surveys.

The evaluation of implementation of different interventions was achieved through a desk-based analysis of HMIS data. The cases and deaths due to infection with *P. falciparum* in children below 5 years of age, from 15 IRS districts and 15 ITNs districts in 2007 and 2008 were collated using the HMIS and data were compared.

2.3.3.3 Sampling

Routine surveillance data from a total of 30 districts were included in the analysis. Among these, fifteen districts solely relied on the deployment of LLINs and the other fifteen districts implemented IRS as the frontline intervention. The study monitored the impact of these interventions on malaria cases, deaths and case fatality rates in children below the age of five years.

2.3.3.4 Statistical design

Malaria cases, deaths and case fatality rates in the selected districts were computed from 2007 to 2008. The chi-square statistic was used to show any change in the parameters between the two years. To assess the epidemiological impact of the two interventions, the odds ratio of malaria cases, deaths and case fatality rates for 2008 relative to 2007 was calculated.

2.4 Ethics clearance

Ethical clearance for this study was sought from the University of Zambia Biomedical Ethical Committee (Assurance No. FWA00000338, IRB00001131 of IOR G0000774 reference code 002-07-07).

CHAPTER THREE

Epidemiological Impact Evaluation of Malaria Control Programme Interventions in Zambia

3.1 Introduction

Measuring the impact of malaria control on reducing morbidity and mortality of this disease is essential (Hay et al. 2008, Snow et al. 2008). This will assist with targeting vector control to cover people at risk (Nyarango et al. 2006, Noor et al. 2007, Hill 2006, Fegan et al. 2007, Barnes et al. 2005), and improve case management (Barnes et al. 2005, Sutherland et al. 2005) and IPT (Breman and O'Meara 2005, O'Meara et al. 2005) where it is critically needed. Many malaria endemic countries are substantially increasing their control activities (Feachem and Sabot 2007, Hay et al. 2008, WHO 2009), and others considering elimination (WHO 2007, WHO 2006). With this increase in activity there is need to optimize impact evaluation of existing control interventions and determine how best to combine and monitor them (Hay et al. 2008).

The main measures for epidemiological evaluation of malaria are parasitological surveys and case surveillance of the human population (Rogier et al. 2009). The impact of malaria control interventions can be monitored using several epidemiological indices including: parasite prevalence as determined in malariometric surveys (an investigation of selected age-groups of a randomly sampled population to assess the degree of malarial endemicity in a location) (WHO 1963), malaria incidence through a comprehensive surveillance system comprising passive case detection (examination of suspected, usually febrile cases presenting routinely to any point of health services), supported by active case detection (examination of fever cases sought through home visits at regular intervals) (Pull 1972, Molineaux et al. 1988) and morbidity and mortality determined through routine surveillance (Hay et al. 2008).

The ideal measure of impact of malaria control is incidence, however due to poor health information systems, reporting and confirmed diagnosis this data is often unavailable (McKenzie et al. 2003, Zurovac et al. 2006, O'Meara et al. 2007, Metselaar and Van Thiel 1959, WHO 1963). Population based household surveys, such as the malaria indicator surveys (W.H.O 2003) have become routine to monitor malaria control interventions coverage and parasite prevalence (Keating et al. 2009, Guerra et al. 2007). These have been made easier with the development of

good quality rapid diagnostic tests (Bell and Peeling 2006, Moody 2002). While sampling of the 2 to 10 years age-group having been shown to be optimal (Smith et al. 2007) and that age standardization techniques can be applied to help compare malaria parasite prevalence surveys across different age cohorts, monitoring the impact with repeated annual parasite prevalence through representative malaria indicator surveys is essential (Brooker et al. 2006, Eliades et al. 2006, Kolaczinski et al. 2005).

A chronological history of malaria control in Zambia (formally Northern Rhodesia from 1911-1964) indicates consistent implementation of different interventions to control malaria over several decades (Table 1.1) with significant success (Utzinger et al. 2001, Utzinger et al. 2002, Sharp et al. 2002). Currently, the National Malaria Control Programme (NMCP) in Zambia implements an integrated approach, consisting of vector control with IRS and ITNs, treatment with ACT, and IPT for pregnant women. This project carried out annual parasitaemia surveys and the impact on infant morbidity and mortality are used to assess the effectiveness of these control measures.

3.2 Results

3.2.1 Programmatic Progress

Chanda et al, (2008) reported the detailed processes implemented by the successful vector control programme in Zambia. Data from population-based surveys and HMIS indicate an increase in the deployment of intervention over the study period. By 2008, 6.1 million LLINs, enough to protect 96% (N = 12.6 million) of Zambia's population, had been distributed country-wide (MoH 2008, CSO 2000). Nationally, representative household surveys indicated an increase in household ITN ownership and utilization by children under the age of 5 years from 44% and 23% in 2006 to 62% and 41% respectively by 2008. Implementation of IRS protected 5.7 million people (approximately 47% of the population) in 2008 with an average coverage of 90.4% of over 1.0 million targeted households, mostly in urban areas (MoH 2008).

3.2.2 Epidemiological Impact of Interventions

3.2.2.1 Routine Surveillance Data in Children <5 years old

Findings from the analysis of HMIS data indicate that a total of 1,679,118 cases of malaria in children below the age of five years were confirmed during routine surveillance, either by using RDTs or microscopy, in 30 of the 72 districts in Zambia between 2007 and 2008. Of these cases, 2,448 deaths due to malaria occurred, with a combined case fatality rate (CFR) of 30.2% (95% CI = 29.87-30.51).

In 2007 alone, 991,722 children had malaria confirmed, resulting in 1,786 deaths with a CFR of 34.6% (95% CI = 34.22-35.04). During the following year, 687,396 children had malaria in the same districts, with 662 deaths reported resulting in a CFR of 22.7% (95% CI = 22.19-23.13). The number of deaths from malaria in this age group in the 30 districts reduced from 2007 to 2008 by 62.9% (95% CI = 60.69-65-17), with the number of cases in the same period reducing by 30.7% (95% CI = 30.60-30.78) and the case fatality rate dropped by 61.6% (95% CI = 60.87-62.31).

There was substantial inter-district heterogeneity in the number of recorded malaria related deaths and case fatality rates (CFR) across the study period. The overall mortality rate in 2007 was 62.0% (95% CI = 60.27-63.81) with the number of deaths ranging from 3 in Kazungula to 507 in Ndola. In 2008, the average mortality in the same 30 districts was lower at 44.1% (95% CI = 41.54-46.56) with the number of deaths ranging from 1 at Kazungula to 83 in Kitwe (Table 3.2). Overall, the odds ratio (OR) for 2007 compared to 2008 was 0.48 (95% CI = 0.42-0.54, P = 0.082) for deaths and 0.55 (95% CI = 0.54-0.57, P = 0.116) for CFR with substantial variations in reductions between IRS and ITN districts (Table 3.1 and 3.2).

The mean mortality in IRS districts was 63.4% (95% CI = 61.25-65.49) compared with 59.1% (95% CI = 55.83-62.29) in ITN districts (P = 0.698) in 2007. The following year, the mean deaths due to malaria in IRS implementing districts and ITN deploying districts was 38.7% (95% CI = 35.66-41.72) and 54.5% (95% CI = 50.20-58.86) respectively (P = 0.102). Overall odds ratio for deaths comparing 2007 and 2008 was 0.37 (95% CI = 0.31-0.43, P = 0.015) in IRS and 0.83 (95% CI = 0.67-1.04, P = 0.666) in ITN districts (Table 3.3). The change in mortality was significant in eight districts, five ITN districts: Luangwa, Sesheke, Namwala,

Chadiza and Chavuma, and three IRS districts; Livingstone, Kazungula and Lusaka (Table 3.1 and 3.2).

In 2007, the average CFR in IRS districts was 50.3 (95% CI = 49.71-50.97) compared with 20.0% (95% CI = 19.55-20.51) in ITN districts (P = 0.0003). In 2008, the average malaria CFR in IRS and ITN implementing districts was 25.8 (95% CI = 25.10-26.48) and 19.3 (95% CI = 18.64-19.94) respectively (P = 0.333). The overall OR for the CFR comparing 2007 and 2008 was 0.34 (95% CI = 0.33-0.36, P = 0.005) in IRS and 0.96 (95% CI = 0.91-1.00, P = 0.913) in ITN districts respectively (Table 3.3). The change in CFR was statistically significant in seven districts, three ITN districts: Chadiza, Kalabo and Luangwa, and four IRS districts: Livingstone, Lusaka, Ndola and Solwezi (Tables 3.1 and 3.2).

In IRS implementing districts the number of deaths and cases reduced by 69.5% (95% CI = 66.93-72.01) and 26.8% (95% CI = 26.73-26.95) respectively from 2007 to 2008, albeit with great inter-district variation. In ITN deploying districts the number of deaths and cases declined by 47.2% (95% CI = 42.97-51.51) and 37.1% (95% CI = 36.94-37.26) from 2007 to 2008 (Table 3.1). There was no statistical significance in the overall odds ratio of cases of malaria for 2008 relative to 2007, in children below the age of five years obtained from routine surveillance data in 30 districts (P = 0.944). There was a significant difference in the reduction of deaths between IRS and ITNs (P = 0.04) than in the reduction of cases between the two interventions (P = 0.198) from 2007 to 2008.

Table 3.1: Deaths due to infection with *Plasmodium falciparum* and malaria case fatality rates in children < 5 years of age, observed during routine surveillance in 15 ITN districts in 2007 and 2008 in Zambia

Sentinel site	ITN Coverage %	Deaths from malaria, (%)(n)(95% CI)		*P*	Case Fatality Rate,(%) (n)(95% CI)		*P*
		2007	2008		2007	2008	
Chadiza	61-80	75.6(41) [62.47-88.75]	35.3(17) [12.57-58.01]	0.00013*	12.7(2445) [11.40-14.04]	3.7(1604) [2.76-4.60]	0.026*
Chama	61-80	67.1(82) [56.90-77.24]	57.8(45) [43.35-72.21]	0.406	14.4(3809) [13.29-15.53]	17.2(1514) [15.27-19.07]	0.619
Chavuma	> 80	80.0(5) [44.94-115.06]	37.5(8) [3.95-71.05]	0.0009*	4.2(942) [2.96-5.54]	10.6(282) [7.04-14.24]	0.096
Chibombo	> 80	57.9(38) [42.19-73.59]	68.4(19) [47.52-89.32]	0.35	19.7(1115) [17.39-22.09]	21.7(599) [18.40-25.00]	0.756
Chinsali	> 80	67.7(127) [59.59-75.85]	57.6(33) [40.72-74.44]	0.367	25.5(3379) [24.04-26.98]	20.7(917) [18.10-23.34]	0.48
Kalabo	> 80	34.7(49) [21.36-48.02]	44.9(49) [30.97-58.83]	0.253	27.1(629) [23.61-30.59]	12.5(1754) [10.99-14.09]	0.020*
Kalomo	61-80	50.0(88) [39.55-60.45]	58.7(46) [44.47-72.93]	0.404	48.0(916) [44.79-51.27]	32.4(834) [29.19-35.55]	0.082
Luangwa	100	58.5(41) [43.46-73.62]	22.7(22) [05.22-40.24]	0.00007*	43.6(551) [39.42-47.70]	12.6(396) [09.36-15.90]	0.00004*
Namwala	> 80	42.9(35) [26.46-59.26]	72.7(11) [46.41-99.05]	0.006*	23.0(653) [19.74-26.20]	47.6(168) [40.07-55.17]	0.0034
Nyimba	61-80	70.7(92) [61.34-79.96]	55.4(56) [42.32-68.38]	0.173	40.5(1604) [38.12-42.92]	30.7(1010) [27.85-33.53]	0.246
Milengi	61-80	70.0(10) [04.60-98.40]	83.3(12) [62.24-104.42]	0.283	8.6(815) [06.67-10.51]	18.4(545) [15.10-21.60]	0.059
Mwinilunga	61-80	60.0(50) [46.42-73.58]	59.6(52) [46.28-72.96]	0.975	10.5(2869) [09.37-11.61]	15.0(2061) [13.45-16.53]	0.373
Samfya	> 80	51.2(162) [41.07-56.47]	55.6(90) [45.29-65.83]	0.671	24.1(3438) [22.68-25.54]	31.1(1610) [28.86-33.38]	0.346
Sesheke	> 80	63.0(27) [44.74-81.18]	37.5(16) [13.78-61.22]	0.011*	12.3(1385) [10.54-14.00]	44.8(134) [36.36-53.20]	0.00002
Zambezi	> 80	69.0(42) [55.07-83.03]	74.1(27) [57.54-90.60]	0.0008*	15.5(1869) [13.88-17.16]	21.5(929) [18.89-24.17]	0.324
All	95	59.1(889) [55.83-62.29]	54.5(486) [50.20-58.86]	0.666	20.0(26419) [19.55-20.51]	19.3(14357) [18.64-19.94]	0.913

*Change since 2007 was significant

Table 3.2: Deaths due to infection with *Plasmodium falciparum* and malaria case fatality rates in children < 5 years of age, observed during routine surveillance in 15 IRS districts in 2007 and 2008 in Zambia

	IRS Coverage (%)		Deaths from malaria, (%)(n)(95% CI)			Case Fatality Rate, (%) (n)(95% CI)		
Sentinel site	2007	2008	2007	2008	*P*(2007-2008)	2007	2008	*P*(2007-2008)
Chililabombwe	95	88	45.0(20) [23.20-66.80]	33.3(12) [6.66-60.0]	0.186	13.8(544) [10.89-16.69]	20.4(196) [14.77-26.05]	0.259
Chingola	97	94	32.6(43) [18.55-46.57]	44.0(25) [24.54-63.46]	0.193	9.6(1446) [8.12-11.14]	15.6(706) [12.90-18.26]	0.232
Chongwe	100	88	62.5(56) [49.82-75.18]	61.5(13) [35.09-87.99]	0.929	27.8(1260) [25.31-30.25]	19.3(414) [15.52-23.12]	0.216
Kabwe	80	97	38.6(57) [25.96-51.24]	31.5(92) [22.03-41.01]	0.397	19.6(1123) [17.27-21.91]	30.8(943) [27.80-33.70]	0.115
Kafue	96	80	40.6(32) [23.61-57.65]	41.9(31) [24.57-59.31]	0.888	14.2(913) [11.97-16.51]	20.6(630) [17.47-23.79]	0.278
Kalulushi	93	93	27.9(43) [14.50-41.32]	63.6(11) [35.21-92.07]	0.0002*	11.5(1045) [9.55-13.41]	10.3(682) [7.98-12.54]	0.797
Kazungula	95	83	42.9(7) [6.20-79.52]	100(1) […-…]	<0.0001*	21.9(137) [14.97-28.83]	28.6(35) [13.60 -43.54]	0.346
Kitwe	103	94	57.8(36) [36.47-69.09]	46.1(180) [38.83-53.39]	0.251	12.9(1468) [11.16-14.58]	73.6(1127) [71.08-76.22]	<0.0001*
Livingstone	94	94	37.0(54) [24.16-49.92]	16.7(12) [4.42-37.76]	0.0056*	48.1(416) [43.28-52.88]	17.7(113) [10.66-24.74]	0.0002*
Luashya	93	87	50.0(58) [37.13-62.87]	50.0(66) [37.94-62.06]	1	36.6(792) [33.26-39.38]	49.8(663) [45.96-53.58]	0.156
Lusaka	94	99	64.6(650) [60.94-68.30]	20.7(270) [15.90-25.58]	<0.0001*	155.4(2703) [62.91-65.81]	18.2(3075) [16.85-19.57]	<0.0001*
Mazabuka	100	95	38.1(113) [29.10-47.00]	43.2(44) [28.54-57.82]	0.572	16.5(2602) [15.06-17.92]	21.0(905) [18.34-23.64]	0.462
Mufulira	91	91	31.8(44) [18.06-45.58]	35.2(54) [22.45-47.93]	0.678	18.7(747) [15.94-21.54]	22.8(833) [19.96-25.66]	0.525
Ndola	90	90	76.6(662) [73.36-79.82]	62.4(157) [54.84-70.00]	0.228	78.4(6468) [77.39-79.39]	25.9(3791) [24.51-27.29]	<0.0001*
Solwezi	86	83	67.8(115) [59.29-76.37]	63.0(27) [44.74-81.18]	0.675	26.9(2895) [25.29-28.53]	12.1(1407) [10.38-13.78]	0.018*
All	93.8	90.4	63.4(1990) [61.25-65.49]	38.7(995) [35.66-41.72]	0.015*	50.3(24559) [49.71-50.97]	25.8(15520) [25.10-26.48]	0.005*

*Change since 2007 was significant

Table 3.3: Odds ratio of malaria cases, deaths and CFR for 2008 relative to 2007, in children < 5 years of age obtained from routine surveillance data in 30 districts, analyzed by vector control intervention type in Zambia

Intervention	Deaths in 2007 (95%CI)%	Deaths in 2008 (95%CI)%	Odds ratio (95% CI)%	*P*
IRS	63.4[61.25-65.49]	38.7[35.66-41.72]	0.37[0.31-0.43]	0.015*
ITN	59.1[55.83-62.29]	54.5[50.20-58.86]	0.83[0.67-1.04]	0.666
All	62.0[60.27-63.81]	44.1[41.54-46.56]	0.48[0.42-0.54]	0.082
Intervention	Cases in 2007 (95%CI)%	Cases in 2008 (95%CI)%	Odds ratio (95% CI)%	P
IRS	49.1[48.77-49.33]	48.3[48.20-48.40]	0.97[0.97-0.98]	0.933
ITN	49.9[49.77-49.99]	49.4[49.21-49.49]	0.98[0.97-0.99]	0.956
All	49.4[49.14-49.58]	48.7[48.39-48.91]	0.97[0.97-0.98]	0.944
Intervention	Case Fatality Rates in 2007 (95%CI)%	Case Fatality Rates in 2008 (95%CI)%	Odds ratio (95% CI)%	P
IRS	50.3[49.71-50.97]	25.8[25.10-26.48]	0.34[0.33-0.36]	0.005*
ITN	20.0[19.55-20.51]	19.3[18.64-19.94]	0.96[0.91-1.00]	0.913
All	34.6[34.22-35.04]	22.7[22.19-23.13]	0.55[0.54-0.57]	0.116

*Confidence interval

Table 3.4: Progress of malaria control in Zambia from 2001 to 2008

Indicator	DHS 2001/2002	MIS 2006	DHS 2007	MIS 2008	*P* (2006-2008)
Percentage of households with at least one ITN	13.6	37.8	53.3	62.3	0.014*
Percentage of households covered with ITN or recent IRS	N/A	43.2	N.A	65.5	0.032*
Percentage of children ages 0-59 months who slept under an ITN the previous night	6.5	24.3	28.5	41.1	0.038*
Percentage of children ages 0-59 months with malaria parasitaemia	N/A	22.2	N/A	10.2	0.035*

*Confidence interval

3.2.2.2 Malaria Prevalence in Children 1 to < 15 years old

A total of 1,823 children aged between 1 and <15 years were tested for *P.falciparum* parasitaemia in all sentinel sites except Manueli and Nyamankalo (Figure 2.2) at the end of the peak malaria transmission periods in April/May 2008. Follow up surveys were carried out including 2,255 children in 2009 and 2,220 children in 2010 in the same period at the same sentinel sites.

Data from two sites were not included in the comparison of 2009 data with baseline because they were either not surveyed in 2008 or they were surveyed during other studies that had different sampling criteria.

The combined prevalence of infection with *P. falciparum* in children 1 to < 15 years of age in 2008 across all sites was 6.8% (95% CI = 5.6 – 8.0) with prevalence ranging from 0% at Myooye to 23.1 at Rufunsa. In 2009, the overall prevalence in children 1 to < 15 years of age for the same 17 sites had decreased to 4.9% (*P* = 0.58) with infection ranging from 0% at Kabulongo, Kafue estates, Mwanachingwala, Mufweshya, Munenga and Myooye to 40.7% at Rufunsa (Table 3.5). However, in 2010 the average prevalence of infection in children 1 to <15 years of age for the same sites had increased to 6.8% (*P*=0.578) with prevalence ranging from 0% at Munenga to 58.2% at Rufunsa.

The reductions in prevalence of infection between 2008 and 2009 were significant in two IRS sites; Kabulongo and Mufweshya, and two ITN sites Chibombo and Mulungushi. The increase in prevalence of infection in 2010 relative to 2009 was significant in two IRS sites Kabulongo (*P* = 0.032) and Kafue estates (*P* = 0.054). There was substantial inter-site heterogeneity in prevalence, particularly in 2008 (Table 3.5).

The overall odds ratio (OR) for prevalence of infection comparing 2008 with 2009 was 0.71 and remained the same for the comparison between 2009 and 2010 (Table 3.6). There was considerable variation in reductions between IRS and ITNs sites (Table 3.6 and Figures 3.3 and 3.4).

In 2008, no significant difference (*P*>0.05) was observed in the combined prevalence in IRS 6.0 % (95% CI = 4.1 – 7.9) compared to ITN sites with 7.2% (95% CI = 5.8 – 8.7). In 2009, there was significant difference (*P* = 0.015) in average prevalence of infection between IRS sites with 0.2% compared to 6.5% at ITN sites (Table 3.6). The mean prevalence in IRS localities was 4.0% compared to 7.6% for ITN sites, which was not significantly different (*P* = 0.291) in 2010. Overall the odds ratio for prevalence of infection comparing 2008 with 2009 was 0.03 (*P* = 0.02) in IRS areas and 0.89 (*P* = 0.85) in ITN areas respectively (Table 3.6). Odds ratios for comparing 2009 and 2010 was 0.04 for IRS (*P* = 0.064) and 0.84 for ITNs (*P* = 0.769).

There was also considerable inter-site variation in reported levels of vector control protection, particularly ITNs and IRS in the three annual surveys (Figure 3.1). Overall intervention effect on prevalence of infection was considerably stronger in IRS treated areas than in ITN ones. There was an incremental effect of using both IRS and ITNs in reducing the prevalence of infection in children <14 years in 2008, 2009 and 2010 (Figure 3.2).

Marked heterogeneity in the utilization of interventions by children aged 1 to <15 years was also observed. The use of ITNs increased from 37.3% in 2008 to 42.0% in 2009 but reduced to 34.6% in 2010 (Table 3.7). This was the trend in most ITNs sites except for Chiawa and Mulungushi which increased over the three years with Chibombo showing the biggest increases from 19.4% in 2008 to 37% in 2009 and 81% in 2010. Comparing 2008 and 2009, there was increased protection of children by IRS (OR = 0.68) but this reduced between 2009 and 2010 (OR = 1.26). The IRS coverage of households with children increased from 2008 to 2009 but reduced in 2010 in Mufweshya, Kabulongo and Mukobeko IRS sites, but not in Kafue estates which showed a steady reduction in IRS coverage from 87.9% in 2008 to 76.7% in 2009 and to 71.4% in 2010 (Table 3.7).

The prevalence of infection in children whose house had not been sprayed in the past year and did not sleep under a net the night before the survey was 6.8%. Children who slept under a net, but whose house had not been sprayed during the past year had a prevalence of infection of 5.2%. Children whose house had been sprayed during the past year, but did not sleep under a net had a significantly lower prevalence of infection of 3.2%. Children who slept under a net in a dwelling that had been sprayed had the lowest risk of infection with a prevalence of 2.6%.

The prevalence of infection varied substantially by age in 2009 and 2010 relative to 2008, with age-specific prevalence being greater in children between 1 and 5 years of age. The reduction in prevalence of infection in children less than five years of age between 2008 and 2009 (OR = 0.48, 95% CI = 0.29-0.78) was greater than that for older children (OR = 0.75, 95% CI = 0.53-0.99), there was a significant difference between intervention effects (P = 0.015). However, between 2009 and 2010, there was an increase in prevalence of infection in both children less than five

years of age (OR = 1.50, 95% CI = 0.91-2.49, *P* = 0.505) and older children (OR = 1.38, 95% CI = 1.02 = 1.87, *P* = 0.578), with no significant difference between the two age groups (*P* > 0.05) suggesting either reduced coverage or reduced efficacy of the interventions.

Figure 3.1: Prevalence of infection in children 1to < 15 years of age in Zambia by reported vector control intervention in 2008, 2009 and 2010 annual surveys

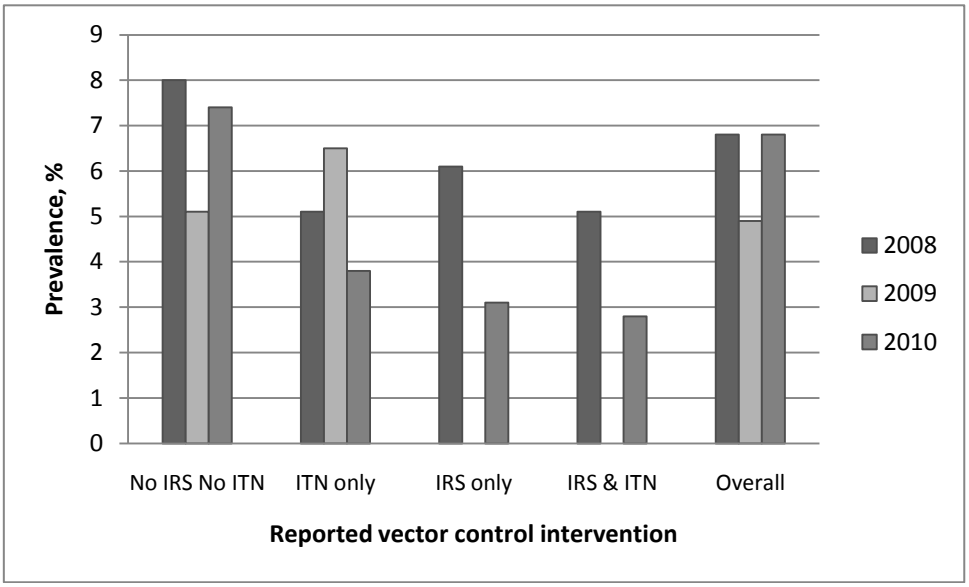


Figure 3.2: Prevalence of infection in children 1 to < 15 years of age in Zambia by reported vector control intervention (2008, 2009 and 2010 combined)

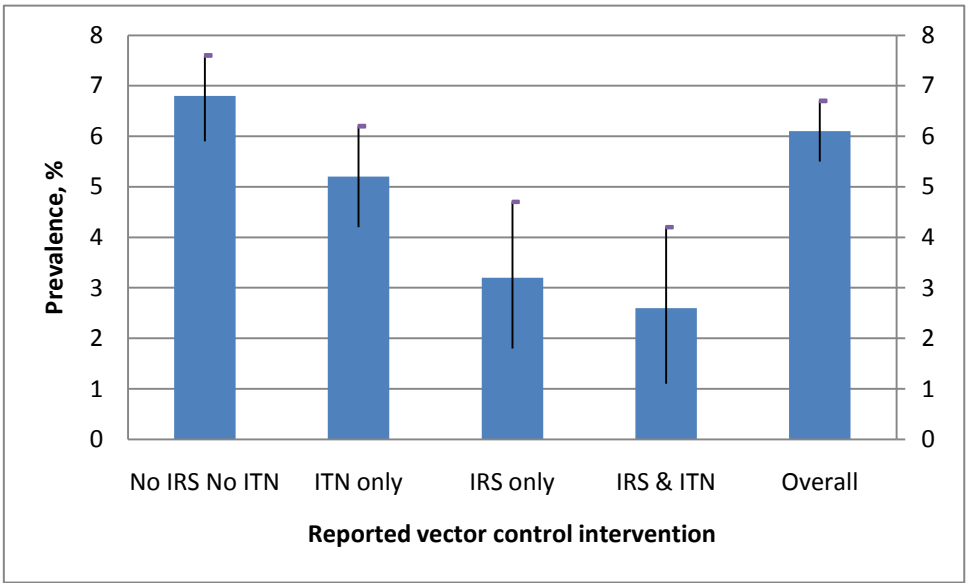


Table 3.5: Prevalence of infection with *Plasmodium falciparum* in children 1 to < 15 years of age, by sentinel site, observed during household surveys in 2008, 2009 and 2010 in Zambia

	April/May 2008		April/ May 2009		April/ May 2010			
Sentinel site	Prevalence of infection,% (n)	95% Confidence interval	Prevalence of infection,% (n)	95% Confidence interval	Prevalence of infection,% (n)	95% Confidence interval	*P*(2008-2009)	*P*(2009-2010)
Chiawa	2 (148)	[0.5 - 8.1]	3.7 (134)	[1.4 - 9.5]	5.1 (136)	[2.3 - 11.2]	0.476	0.637
Chibombo	21.2 (146)	[12.7 - 33.3]	9.3 (161)	[4.5 - 18.2]	3 (132)	[1.3 - 7.1]	0.0311*	0.072
Chikankata	1.1 (93)	[0.2 - 7.2]	0.7 (147)	[0.1 - 4.9]	0.7 (136)	[0.1 - 4.7]	0.765	1
Chimoto[a]	3.2 (93)	[0.8 - 12.8]	0.7 (141)	[0.1 - 5]	3.4 (145)	[1.1 - 9.9]	0.206	0.182
Chipepo	11 (73)	[6.2 - 18.6]	4.1 (123)	[1.5 - 10.7]	5 (120)	[2.3 - 10.3]	0.076	0.766
Chisamba	0.9 (109)	[0.1 - 6.3]	0.7 (139)	[0.1 - 5.1]	2 (150)	[0.7 - 5.9]	0.874	0.429
Chobana	8.9 (79)	[3.5 - 20.5]	3.2 (124)	[0.7 - 13.2]	1 (97)	[0.1 - 7]	0.101	0.283
Chunga	3.6 (83)	[0.5 - 20.7]	4.2 (95)	[1.5 - 11.6]	1.9 (104)	[0.3 - 12.1]	0.83	0.352
Kabulongo[a]	11.4 (158)	[10.9 - 36.5]	0 (84)	-	4.6 (130)	[1.6 - 12]	0.0007*	0.032*
Mukobeko[a]	7 (157)	[3.3 - 14.1]	6.7 (134)	[3.1 - 13.8]	6.2 (130)	[3.2 - 11.4]	0.933	0.89
Kafue estate[a]	2.3 (128)	[0.8 - 6.6]	0 (116)	-	3.7 (137)	[1.4 - 9.1]	0.129	0.054*
Mwanachingwala	1.2 (86)	[0.2 - 7]	0 (152)	-	1.5 (131)	[0.2 - 10.6]	0.273	0.221
Mufweshya[a]	4.3 (69)	[1 - 17.1]	0 (73)	-	1.8(113)	[0.5 - 6.6]	0.038*	0.18
Mulungushi	15.2(46)	[7.8 - 27.5]	4.6 (131)	[1.5 - 13.2]	8.1 (123)	[3.8 - 16.5]	0.0172*	0.326
Munenga	1.5 (134)	[0.4 - 5]	0 (138)	-	0 (134)	-	0.22	0
Myooye	0 (117)	-	0 (140)	-	3 (133)	[0.9 - 9.2]	-	0.083
Rufunsa	23.1 (104)	[11.6 - 40.6]	40.7 (135)	[30.1 - 52.4]	58.2 (141)	[46.5 - 69]	0.0275*	0.078
All	6.8 (1823)	[5.6 - 8.0]	4.9 (2167)	[4.0 - 5.8]	6.8 (2192)	[5.7-7.8]	0.578	0.578

*Change since 2008 was statistically significant; [a]IRS sites

Table 3.6: Odds ratio of infection with *Plasmodium falciparum* for 2009 relative to 2008, in children 1 to < 15 years of age obtained from household surveys conducted at 17 sentinel sites, by vector control in Zambia

Intervention	Prevalence in 2008 (95%CI)%	Prevalence in 2009 (95%CI)%	Prevalence in 2010 (95%CI)%	2008-2009 Odds ratio (95% CI)%	2009-2010 Odds ratio (95% CI)%	*P*(2008-2009)	*P*(2009-2010)
IRS	6.0[4.11 - 7.89]	0.2[0.18 - 0.88]	4.0[2.47 - 5.47]	0.03[0.00 - 0.21]	0.04[0.01 - 0.32]	0.0198*	0.064
ITN	7.2[5.77 - 8.67]	6.5[5.31 - 7.71]	7.6[6.30 - 8.94]	0.89[0.67 - 1.20]	0.84[0.64 - 1.11]	0.85	0.769
All	6.8[5.64 - 7.96]	4.9[3.98 - 5.80]	6.8[5.70 - 7.80]	0.71[0.54 - 0.92]	0.71[0.54 - 0.92]	0.578	0.578

*Confidence interval

Table 3.7: Reported protection through ITN utilization and IRS coverage by children 1 to < 15 years of age at sentinel sites

	ITN use (%)(n)(95% CI)			Odds ratio (OR)(95% CI)		IRS Coverage (%)(n)(95% CI)			Odds ratio (OR)(95% CI)	
Sentinel site	2008	2009	2010	(2008-2009)	(2009-2010)	2008	2009	2010	(2008-2009)	(2009-2010)
Chiawa	37(165) [26.3,49.1]	52.5(141) [35.8,68.6]	56.1(139) [40.9,70.3]	1.88 (1.19-2.98)	1.16 (0.72-1.85)	8.3(157) [2.8,21.8]	0	0	0	0
Chibombo	19.4(155) [11.1,31.6]	37(165) [25.0,50.8]	81.3(134) [63.6,91.6]	2.44 (1.47-4.06)	7.43 (4.34-12.72)	0	6.7(165) [2.0,20.0]	6.7(134) [1.0,35.0]	0	1.01 (0.41-2.51)
Chikankata	41(100) [25.3,58.8]	34.2(152) [17.8,55.5]	21.4(145) [9.8,40.5]	0.74 (0.45-1.26)	0.52 (0.31-0.88)	5(100) [0.7,28.2]	0	46.9(145) [23.9,71.3]	0	0
Chimoto	25.2(103) [13.9,41.5]	26.1(142) [12.5,46.4]	12.8(148) [6.1,25.1]	1.04 (0.58-1.87)	0.42 (0.23-0.80)	3.9(103) [0.5,23.1]	0	55.6(144) [33.8,75.4]	0	0
Chipepo	37(77) [19.6,60.0]	27.2(125) [14.4,45.3]	43.2(125) [30.8,56.6]	0.62 (0.34-1.13)	2.04 (1.20-3.46)	6.5(77) [0.9,34.6]	0	0	0	0
Chisamba	27.7(112) [17.1,41.5]	19.9(141) [11.7,31.7]	45(151) [32.9,57.8]	0.65 (0.36-1.16)	3.31 (1.96-5.58)	2.7(112) [0.4,16.8]	1.5(136) [0.2,9.9]	0	0.54 (0.09-3.30)	0
Chobana	51.8(83) [30.1,72.8]	57.3(124) [38.1,74.5]	15.8(101) [8.4,28.0]	1.25 (0.71-2.18)	0.14 (0.07-0.27)	0	0	0	0	0
Chunga	47.7(88) [31.2,64.7]	54.1(98) [36.4,70.8]	41.1(112) [25.8,58.3]	1.29 (0.73-2.30)	0.59 (0.34-1.02)	0	0	0	0	0
Kabulongo	54.3(164) [46.7,61.9]	43.5(92) [28.6,59.6]	44.6(130) [27.0,63.7]	0.65 (0.39-1.08)	1.05 (0.61-1.79)	73.9(153) [66.9,80.8]	83.7(92) [56.1,95.4]	21.5(130) [10.0,40.5]	1.82 (0.94-3.52)	0.05 (0.03-0.11)
Kabwe	32.4(170) [21.6,45.4]	41.2(136) [27.8,56.0]	37(135) [26.1,49.5]	1.46 (0.92-2.34)	0.84 (0.52-1.37)	29.4(170) [17.4,45.2]	39.7(136) [23.1,59.1]	7.4(135) [1.1,37.5]	1.58 (0.98-2.55)	0.12 (0.06-0.25)
Kafue estate	30.6(134) [18.4,46.3]	36.2(116) [24.0,50.5]	20.7(145) [11.3,34.8]	1.29 (0.76-2.18)	0.46 (0.27-0.80)	87.9(132) [71.5,95.4]	76.7(116) [55.9,89.5]	71.4(119) [48.3,87.0]	0.46 (0.23-0.90)	0.76 (0.42-1.36)
Mwanachingwala	28.9(90) [18.3,42.4]	63.2(155) [55.6,70.8]	23.9(134) [5.9,61.0]	4.23 (2.42-7.41)	0.18 (0.11-0.31)	0	0	18.7(134) [2.8,64.6]	0	0
Mufweshya	56.5(92) [41.3,70.6]	54.4(79) [40.4,67.8]	29.4(119) [17.0,45.8]	0.92 (0.50-1.68)	0.52 (0.30-0.92)	56.3(87) [36.9,74.0]	90.8(76) [73.3,97.3]	45.1(113) [26.9,64.7]	7.64 (3.15-18.53)	0.08 (0.04-0.20)
Mulungushi	34(47) [16.9,56.7]	47.1(138) [34.1,60.5]	49.2(132) [33.3,65.4]	1.73 (0.87-3.44)	1.09 (0.68-1.76)	17(47) [4.2,49.1]	2.2(137) [0.3,14.1]	0	0.12 (0.03-0.43)	0
Munenga	38.5(135) [23.3,56.4]	41(139) [23.5,61.1]	16.1(137) [8.3,28.7]	1.11 (0.68-1.80)	0.28 (0.16-0.49)	25.2(135) [10.6,48.9]	4.3(139) [0.6,25.3]	10.2(137) [2.5,33.4]	0.13 (0.05-0.33)	2.52 (0.94-6.77)
Myooye	21.6(125) [10.3,39.8]	29.5(146) [17.4,45.2]	8.6(139) [3.9,17.9]	1.52 (0.87-2.64)	0.23 (0.11-0.45)	0	14.8(142) [5.1,35.8]	30.2(139) [14.6,52.3]	0	2.50 (1.39-4.49)
Rufunsa	58.8(114) [42.4,73.4]	58.5(135) [40.4,74.6]	42.4(144) [29.1,56.8]	0.99 (0.60-1.64)	0.52 (0.32-0.84)	0	2.2(135) [0.3,14.4]		0	0
ALL	37.3(1954) [35.1-39.4]	42(2224) [39.9-44.0]	34.6(2270) [32.7-36.6]	1.22 (1.08-1.38)	0.73 (0.65-0.83)	20.8(1928) [18.9-22.6]	15.2(2205) [13.7-16.7]	17.4(2230) [15.8-18.9]	0.68 (0.58-0.80)	1.26 (1.08-1.48)

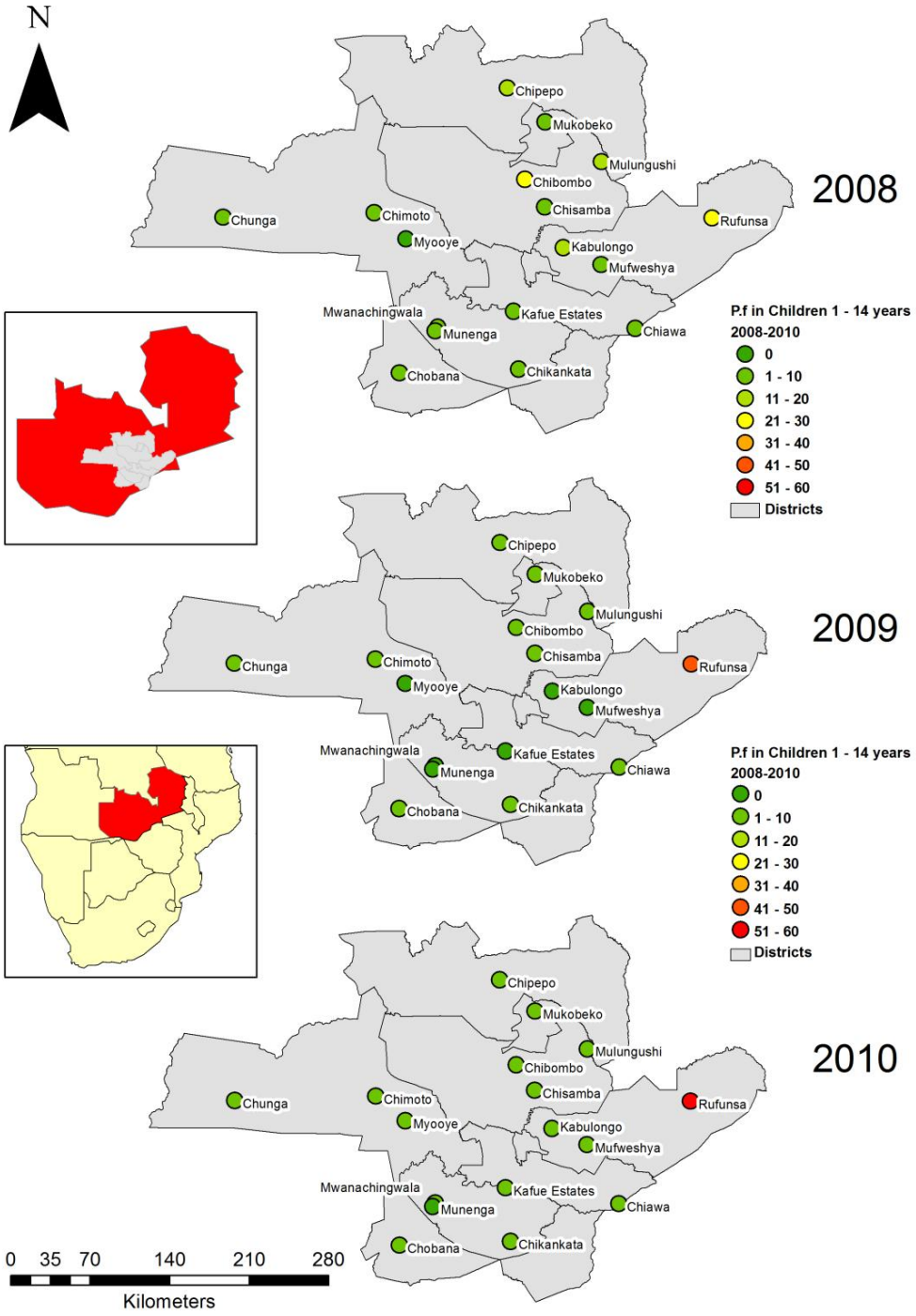


Figure 3.3: *P. falciparum* malaria parasite prevalence in children 1 to < 15 years in monitoring sentinel sites from 2008 to 2010 surveys.

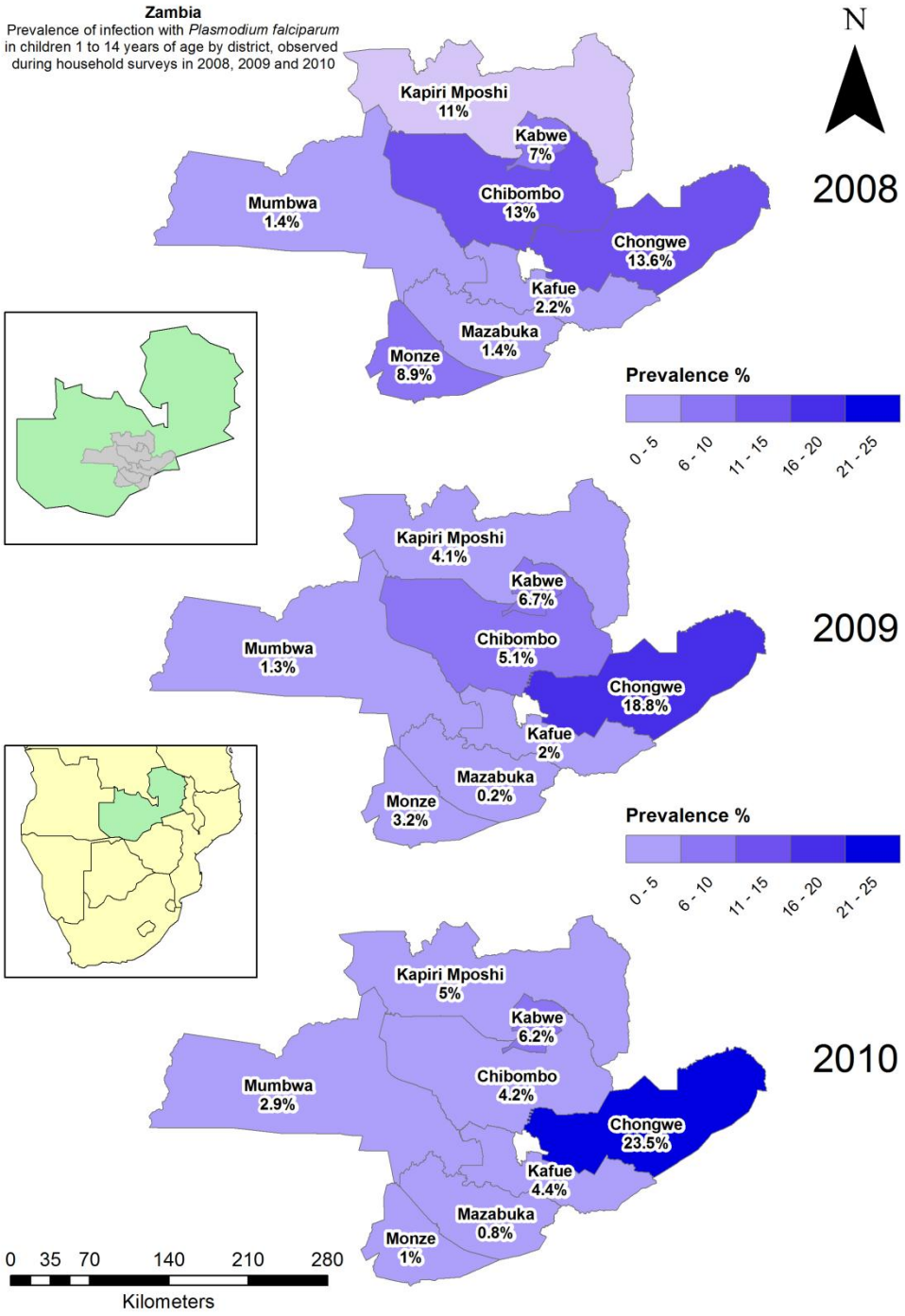


Figure 3.4: Prevalence of infection with *P. falciparum* in children 1 to < 15 years as observed during the annual parasitaemia surveys from 2008 to 2010 by district.

3.3 Discussion

In response to the increasing burden of malaria and the call by the WHO for scaled up implementation of proven vector control interventions (WHO 2007, WHO 2006), coupled with the unprecedented availability of resources for vector control, the Zambian National Malaria Control Programme (NMCP) has made progress in setting up strategies, scaling up programmatic implementation of interventions and monitoring their epidemiological impact on malaria control (Chanda et al. 2008, WHO 2004).

Since malaria transmission is endemic in most of the country IRS and ITNs have been co-implemented, in accordance with clearly defined eligibility criteria. IRS was targeted predominantly at urban and peri-urban areas and ITNs at rural areas. These interventions have been systematically scaled up (Figures 1.3 and 1.4). The national coverage of both ITNs and IRS has surpassed the international targets of at least 80% of households. While ITN coverage with standard criteria is about 96%, a high proportion of households (59%) do not use a net (MoH 2008). In this study, the low percentage (%) coverage of IRS in sentinel sites within non-IRS districts only reflects pest control activities conducted by the private sector (Table 3.7).

Notwithstanding the fact that the impact of malaria control can be evaluated by repeated population-based surveys; parasite prevalence, malaria–specific mortality and all course mortality, inferences can be drawn from national surveillance reports. However, the potential of routine surveillance data in evaluation studies have not been fully exploited (WHO 2009) given their high variability in quality.

The reported number of malaria cases and deaths from routine surveillance data are used as core indicators for tracking the progress of malaria control programmes (WHO 2009). Continuous reporting reflects changes in the implementation of interventions or climatic changes and routine case and death data is available across the country. However, changes in the numbers of cases and deaths may not necessarily reflect changes in incidence of disease in the population due to inconsistencies in reporting at public and private health facilities, or home treated morbidity and limited definitive diagnosis. These sources of error or bias are

addressed by the country-wide scaling up definitive diagnosis using microscopy in hospitals and clinics and RDTs in rural health centres and health posts, promotion of information, education and communication (IEC) and community based interpersonal communication (MoH 2006) and monitoring of the number of laboratory tests undertaken and trends in the malaria (slides or RDT) positivity rate. As such, data on malaria trends in Zambia is comprehensive with complete HMIS records that are backed up with information from three nationally representative household surveys conducted in 2006, 2008 and 2010 (WHO 2009, MoH 2010).

The relationship between malaria transmission intensity and mortality in Africa has been reviewed by Smith and others (Smith et al. 2001). It is estimated that in sub-Saharan Africa, at least 75 % of deaths ascribed to malaria are in children less than five years of age (Snow et al. 1999, WHO 2003). Dramatic reductions of over 50% in childhood mortalities from malaria and all causes have been reported in settings where high coverage has been reached with effective interventions (WHO 2009). A significant reduction in malaria transmission should therefore have a considerable impact on child mortality in areas where IRS and ITNs have been operationally deployed on a large scale in addition to case management with ACTs.

In this study, the average number of deaths and cases due to malaria in Zambia fell significantly from 2007 to 2008 by 62.9% and 30.7% respectively. During this period, IRS using pyrethroids and DDT was associated with a statistically significant overall reduction in mortality (OR = 0.37, 95% CI = 0.31-0.43, P = 0.015) ITNs not so (OR = 0.83, 95% CI = 0.67-1.04, P = 0.666) in children under the age of five per intervention year.

Routine hospital data, reported through the HMIS, provides a proxy for measuring the incidence of severe malaria and for crudely measuring morbidity rates (de Savigny and Binka 2004). Case fatality rates are known to decrease with age, under-five children are at high risk, with infants being the most vulnerable in sub-Saharan Africa (Ejov et al. 1999, Baird et al. 1998, Murphy and Breman 2001, Reyburn et al. 2005). Children are vulnerable to malaria from about 4 months of age because of reduced maternal immunity, and, in highly endemic areas during the peak transmission season; approximately 70% of one-year-olds have malaria parasites in

their blood (Murphy and Breman 2001, Reyburn et al. 2005). Describing trends and patterns of such data could assist in monitoring and planning resource needs in a health system (Gething et al. 2006).

Case fatality rates from the HMIS were used to assess the effects and impact of malaria vector intervention measures on the severity of malaria between 2007 and 2008. The reduction in case fatality rates varied across the different districts with the greatest reduction occurring in Lusaka ($P < 0.0001$) (Table 3.1 and 3.2). Comparing 2007 and 2008, there was a better intervention effect on CFR in IRS localities (OR = 0.37, 95% CI = 0.33-0.36, $P = 0.005$) than in ITN areas (OR = 0.96, 95% CI = 0.91-1.00, $P = 0.913$). The overall case fatality rate dropped from 2007 to 2008 by 61.7% while the mean CFR dropped by 67.6% in IRS areas compared to a decline of 47.7% in ITN areas. Increase in CFR for children aged 6–14 years has been reported (Kazembe et al. 2006), although these are supposed to be protected through acquired immunity, this may reflect some aspects of health seeking behaviour, and emphasize the need for prompt and effective management of malaria for all children including those aged over five years even if such cases may not frequently occur in the general population (Greenberg et al. 1989, Murphy and Breman 2001).

Parasite prevalence, particularly in biologically vulnerable people, such as young children as measured in prevalence surveys using RDTs is a good indicator of the reservoir of infection in a population (Craig et al. 2002, Kleinschmidt et al. 2006). As such, despite its non linear relationship with transmission intensity, prevalence of infection with malaria parasites in children is widely used as a proxy measure for malaria transmission intensity (Beier et al. 1999).

Data comparing 2008, 2009 and 2010 surveys exhibit strong spatial heterogeneity in parasite prevalence, regardless of the low endemicity in several sentinel sites (Figures 3.3 and 3.4). The mean prevalence of parasite infection in children 1 to < 15 years of age was 6.8% (95% CI = 5.6-8.0) in 2008, 4.9% (95% CI = 4.0-5.8) in 2009 and 6.8% (CI = 5.7-7.8) in 2010. Comparing the change in prevalence of infection between 2008 and 2009 and between 2010 and 2009 gave an odds ratio (OR) of 0.71 (95% CI = 0.54-0.92) which suggests that overall prevalence of malaria infection has stayed the same since 2008. Rufunsa showed a significant

increase in prevalence (23.1 to 40.7 to 58.2%) over the 3 surveys despite 58.8%, 58.5% and 42.4% usage of nets respectively. At Myooye, parasite prevalence was maintained at zero in both 2008 and 2009 population-based parasitaemia surveys when ITN use was at 21.6% and 29.5% respectively but increased to 3% in 2010 when net utilization dropped to 8.6% (Table 3.7). This demonstrates the effectiveness of ITNs in controlling malaria if used correctly.

IRS had a much greater impact compared to the ITNs on parasite prevalence (P = 0.015) regardless of the overall increase observed in 2010 relative to 2009 (Figure 3.1 and 3.2, Table 3.7). However, the increased impact observed in IRS areas could as well be ascribed to the added value of the presence of ITNs acquired through the antenatal and child clinics and through the commercial sector. There was considerable variation in overall reduction in parasite prevalence in children 1 to <15 years between IRS (OR = 0.03, 95% CI = 0.00-0.21) and ITN (OR = 0.85, 95% CI = 0.67-1.20) sites. This study has also demonstrated an incremental mutual protective effect of combined use of IRS and ITNs (Figure 3.1 and 3.2).

Kleinschmidt et al, (2009) reported that household surveys conducted in Bioko, Equatorial Guinea, and in Zambezia, Mozambique provided a strong evidence of the combined protective effect of ITNs and IRS relative to one intervention alone, and concluded that future studies to determine the additional protective value of combined use of IRS and ITNs are needed to ascertain that each intervention is effective on its own in a particular setting by including programme implementation indicators that are adequately and independently monitored (Kleinschmidt et al. 2009, Guerra et al. 2007).

Prevalence of infection varied substantially among children 1 to < 15 years of age across the study sites by type of intervention. The combined prevalence of children who slept under a net was 5.2% compared to 3.2% in children who slept in sprayed houses, Prevalence was much reduced (2.6%) in children who used a net in a sprayed house. There was better intervention effect of IRS than ITNs but with incremental combined effects. However, overall use of both interventions increased from 2008 to 2009 but reduced markedly in 2010. Only ITN sites (Chibombo, Chiawa and Mulungushi) showed a steady increase in coverage and utilization

(Table 3.7). This situation could explain the overall decrease in prevalence from 2008 to 2009 and an increase in 2010 observed in this study.

However, the overall reduction in morbidity and mortality cannot be exclusively ascribed to the two transmission-reducing interventions, as ACTs are concurrently being implemented across the country (Sipilanyambe et al. 2008). The implementation of ACTs is known to contribute significantly to improved cure rates and decreased gametocyte carriage (Barnes et al. 2009, Barnes et al. 2005). Although other studies have reported impact of combined interventions on morbidity and mortality of all age-groups (Nyarango et al. 2006), this study demonstrates the feasibility of monitoring the impact of vector control interventions based on morbidity and mortality in children below the age of five years using routine surveillance data and prevalence in children 1 to < 15 years old.

Children less than five years of age had a lower risk of infection compared with older children across all the sentinel sites. Given the endemicity of malaria transmission in Zambia, a peak of parasite prevalence at a younger age is expected than the prevalence levels detected in children between 5 and 14 years of age (Baird et al. 1998, Kleinschmidt and Sharp 2001). The data in this study indicate that older children between 5 and 14 had a higher prevalence of parasite infection than those aged below five years, implying that younger children had less exposure to infective bites than the older children. This finding corroborates the results observed by Kleinschmidt et al, (2006) in Bioko Island, and warranted additional investigation, further challenges the widely held premise that children below the age of five years are the group at risk (Kleinschmidt et al. 2006).

With evaluation now being complicated by the scaling-up of malaria control, all age-groups are susceptible to clinical malaria, albeit with different levels of risk, but transmission is reduced when interventions are used universally because the chances of the mosquito vectors becoming infected and living long enough to become infective and bite human beings are reduced. This suggests the critical need for evidence based deployment of interventions, particularly for those targeting exclusively the children under the age of five in high transmission areas and covering the whole population, not just the most vulnerable in low transmission

settings (Killeen et al, 2007).

Several studies on the impact of malaria control interventions on morbidity and mortality have been conducted in Zambia either as population based surveys or hospital based routine surveillance with widely heterogeneous results (Sharp et al. 2002, McClean and Senthilselvan 2002, Utzinger et al. 2001, Chanda et al. 2009). Significant reductions of over 50% in malaria cases and deaths in all age groups were reported following the implementation of environmental management strategies on the Copper belt province (Utzinger et al. 2001, Utzinger et al. 2002). Surveys conducted in children under 5 years of age from 2007 to 2008 in Chongwe, a district implementing IRS and ITNs showed 0.7% (n = 1378) cases with no severe case or death recorded (Chanda et al. 2009).

Nationally representative malaria indicator surveys have also been conducted in Zambia (MoH 2006, MoH 2008) and the findings has shown marked reductions in the prevalence of parasite infection between 2006 and 2008 (Table 3.4). The number of in-patient malaria cases and deaths among children < 5 years of age decreased by 57% and 62% respectively (MoH 2008). Similar findings were reported from all causes in children aged 1-59 months during the 2007 demographic health Survey (CSO 2007). In the third and fourth quarter of 2008, surveillance data of malaria in-patient cases and deaths was 55% and 60 % respectively, lower than the averages for 2001 and 2002 (WHO 2009). Results from the present study, largely corroborate the findings of these surveys.

With increased resources, vector control programmes using IRS and ITNs have been successfully implemented in a number of countries in Africa (Nyarango et al. 2006, Mabaso et al. 2004, Sharp et al. 2002, Chanda et al. 2008). Most of these programmes are being monitored and evaluated using clinical and entomological surveys that include parasite prevalence (Kleinschmidt et al. 2006, Sharp et al. 2007). However, this is the first evaluation of the impact of large scale IRS and ITNs on morbidity and mortality in children below the age of five using routine surveillance data in an operational area and the results indicate a marked impact on the two indicators albeit with disparities on the effectiveness of the two interventions.

Although there was an overall reduction in deaths and cases in children <5 years of age, and prevalence in children 1 to <15 years old, there were a number of districts where these indicators remained persistently high. Pin-pointing precisely the factors responsible for persistence of high deaths and cases in these districts could be difficult, as the low impact of ITNs in operational settings could in large part be attributed to the waning ownership, use and net durability (physical and insecticide). Although high coverage was attained during the "catch-up" programme, some nets were distributed as early as 2005. This situation underscores the need for a viable "keep-up" programme to maintain effective high coverage (WHO 2005, Lengeler et al. 2007).

The comparatively high impact observed in IRS districts could be as a result of a combination of both IRS and ITNs, as most rural parts of these districts are also covered with ITNs through the country-wide mass distribution programme. Since the eligibility criteria for deployment prioritizes ITN distribution in all rural areas IRS implementation has encroached into these rural areas in some districts. In urban and peri-urban areas where IRS is confined, the uptake and utilization of anti-natal and child clinic, and commercially distributed ITNs has improved markedly in the wake of enhanced IEC campaigns. This view is further supported by the 2008 malaria indicator survey that ITN coverage in Zambia was similar for the poorest (63%) and richest quintiles (65%) and in urban (59%) and in rural areas (64%). Including, the implementation of larval source management using bio-larvicides and environmental management in urban areas of IRS districts (Masaninga F, unpublished data).

While there was marked heterogeneity in the average deaths recorded in the IRS and ITN areas, there was no statistical difference in the mean number of cases between the two interventions in the two years. This situation could be ascribed to the spatial homogeneity in IRS and ITN coverage.

By April 2009, overall malaria deaths reported from health facilities had declined by 66% in Zambia following scaling up of LLINs and IRS between 2006 and 2008, when malaria deaths declined by 47% and nation-wide surveys showed parasite prevalence declined by 53% (Table 3.4). Although the malaria control programme in

Zambia has made substantial progress in reaching households with LLINs and their use and IRS. It is to be expected that universal coverage for ITN, IRS and ACTs is likely to elicit even more decline in malaria burden. In moderate to low transmission setting countries like Zambia, the RBM target of >75% reduction of malaria burden may be attained even several years before 2015 (WHO 2009).

Vector control is pivotal in reducing the burden of vector-borne diseases, as evidenced by its success in reducing or interrupting disease transmission when coverage is sufficiently high, adding resilience to the public health gains achieved through disease management and giving high priority to prevention (WHO 2008). The decrease in malaria deaths and case fatality rates and cases, as observed through routine surveillance, further strengthens the evidence for the reduction of malaria in Zambia following the scaling up of interventions, as monitored by parasite prevalence during malaria indicator surveys.

Despite the fact that impact continues to be measured by parasite prevalence surveys until *P. falciparum* parasite rate is 5% less, all levels below 10% parasite prevalence imply extremely low transmission and the information provided for control is minimal and the error and numbers required for reliable surveys is maximal (Hay et al. 2008) . As the parasite rate falls below the 10% level, substantial effort should be invested in improving the rigour and depth of active and passive case detection (Molineaux et al. 1988, Pull 1972) . Therefore, the findings in this study justify the strengthening of routine surveillance in these low transmission areas of the country.

The prevalence of any condition is measured from a sample of a reasonably homogeneous population, so that its precision can depend on the sample size and the amount of the disease (Jovani and Tella 2006, Molineaux et al. 1988, Gregory and Blackburn 1991). The confidence we can place in an estimate of prevalence will decrease as the numbers sampled become smaller or as the disease becomes rarer (Hay et al. 2008). However, the reliability of a malaria indicator surveys diminishes with declining prevalence, as the indicators cease to be sensitive enough to measure further progress, when parasite rates have dropped to a level of between 1% and 3% (Yekutiel 1960, WHO. 1971). The population sampled must increase for a specified level of confidence in an estimate to be maintained.

Therefore, the low levels of transmission detected in this study demonstrates the need for comprehensive monitoring and strongly suggests the need for complementing parasite prevalence survey data with routine surveillance system data (Molineaux et al. 1988, Pull 1972) when monitoring the impact of interventions in low transmission settings.

CHAPTER FOUR

Operational Impact of Indoor Residual Spraying and Insecticidal Bed Nets on Malaria Vector Bionomics in Low Transmission Settings of Zambia

4.1 Introduction

In sub-Saharan Africa, high malaria transmission rates are a direct consequence of the excellent vectorial capacity of the three major vectors of the disease; *Anopheles gambiae* s.s, *An. arabiensis* and *An. funestus* (Gillies and Coetzee 1987, Gillies and De Meillon 1968). However, implementation of effective malaria control strategies, including vector control and case management (Bhattarai et al. 2007, Fegan et al. 2007, Sharp et al. 2007) has resulted in decreased malaria transmission in many areas (Guerra et al. 2007, Okiro et al. 2007, Rodrigues et al. 2008, Ceesay et al. 2008, O'Meara et al. 2008). In order to reduce disease transmission more rapidly than is feasible with one method alone, or, to increase overall coverage of vector control protection (Beier et al. 2008) some malaria control programmes have deployed a combination of vector control interventions in the same malaria risk areas (Kleinschmidt et al. 2009)

The impact of malaria on mortality and morbidity are determined by vector-mediated transmission intensity (Molineaux et al. 1988, Lengeler et al. 1997, Beier et al. 1999), and post-inoculation factors that include pre-existing immunity, age, nutrition, genetic background, and access to anti-malarial drugs (Rihet et al. 1998, Modiano et al. 1998, Gilbert and Hill 1998, Trape and Rogier 1996). Determining the geographical vector distribution, monitoring of entomologic risk factors and evaluating the impact of interventions on malaria transmission is essential for effective malaria control program policy development (Okara et al. 2010).

Malaria transmission intensity affects most aspects of malaria ecology, epidemiology and control (Snow et al. 1997, Snow and Marsh 2002) and is a critical determinant of malarial disease burden. Its measurement can help define health problems (Greenwood 2008, Hay et al. 2008, Reyburn et al. 2005). Therefore, to objectively evaluate options for malaria control, a thorough understanding of the ecology and epidemiology of malaria and availability of accurate estimates of malaria transmission intensity are necessary (Smith et al. 2007).

The intensity of malaria transmission can be measured in several ways; Entomological Inoculation Rate (EIR), Parasite Rate, Annual Parasite Index, and

Spleen Rates but only a few are generally used for evaluating control programmes (Shaukat et al. 2010). The current gold-standard for measuring malaria transmission intensity is the EIR, determined as the number of infectious bites per person per year (Killeen et al. 2000). However, many errors emerge in estimating both the human biting rate and the sporozoite rate. These are the result of variation in method used, subjectivity of mosquitoes to the capturer, diligence of the technical teams (Fontenille and Simard 2004) and lack of consistently used standard EIR protocols, including logistical difficulties and ethical issues concerning the human landing catches (Shaukat et al. 2010, Killeen et al. 2000). More recently antibody sero-conversion rates (SCR) have shown a tight correlation with EIR and have facilitated rapid assessment of malaria transmission intensity (Stewart et al. 2009, Drakeley et al. 2005).

Mosquito infection rates, together with concurrent human-landing density data provide parameters for estimating the intensity of transmission and entomological inoculation rates that serve as a relative measure of disease risk among exposed human populations (Killeen et al. 2000). The determination of the presence of malaria sporozoites in wild caught *Anopheles* mosquitoes remains an integral component in understanding the transmission dynamics in area-specific malaria epidemiological studies (Wirtz and Burkot, 1991). The detection of advanced stage sporozoites in mosquitoes also provides compelling evidence to incriminate a vector species (Bangs et al. 2002).

Available evidence indicates that malaria prevalence, incidence, morbidity, and mortality increase with transmission intensity (Molineaux 1997, Lengeler et al. 2007, Beier et al. 1999). As such, they have frequently been used as indicators for impact of control interventions. However, measurable impacts of specific interventions on the vector population, sporozoite rates or infectious reservoir have been observed in the field (Macdonald 1957, Molineaux 1997, Killeen et al. 2000, Protopopoff et al. 2007, Sharp et al. 2007).

Significant scale-up in coverage rates of IRS and ITNs in Zambia over the last ten years mean that vector species composition, densities and sporozoite rates are unlikely to have remained constant. This chapter reports on the monitoring of the

relative index for transmission through species abundance and sporozoite rates over a two years study period.

4.2 Results

4.2.1 Mosquito Collections and Identification of Vector Species

During the period from April 2008 to May 2010, mosquitoes were trapped for 85, 320 nights from 19 sentinel sites (Figure 4.1). Chunga sentinel site was not included in this part of work due to unsuitability of housing structures. A total of 619 *An. gambiae s.l.* and 228 *An. funestus s.l.* were collected and morphologically identified. Five hundred and forty nine *An. gambiae s.l.* were subsequently identified to species level. There were four *An. gambiae s.s,* 199 *An. arabiensis and* 322 *An. quadriannulatus.* Two hundred and four *An. funestus s.l* were identified to species, these were 14 *An. funestus s.s,* 98 *An. parensis*, 20 *An. rivulorum*, 18 *An. leesoni* and 16 *An. vaneedeni* and 14 were identified as the recently described *An. funestus*-like and 23 were unidentified. Only 1 *An. nili s.s* in the *An. nili* group was identified.

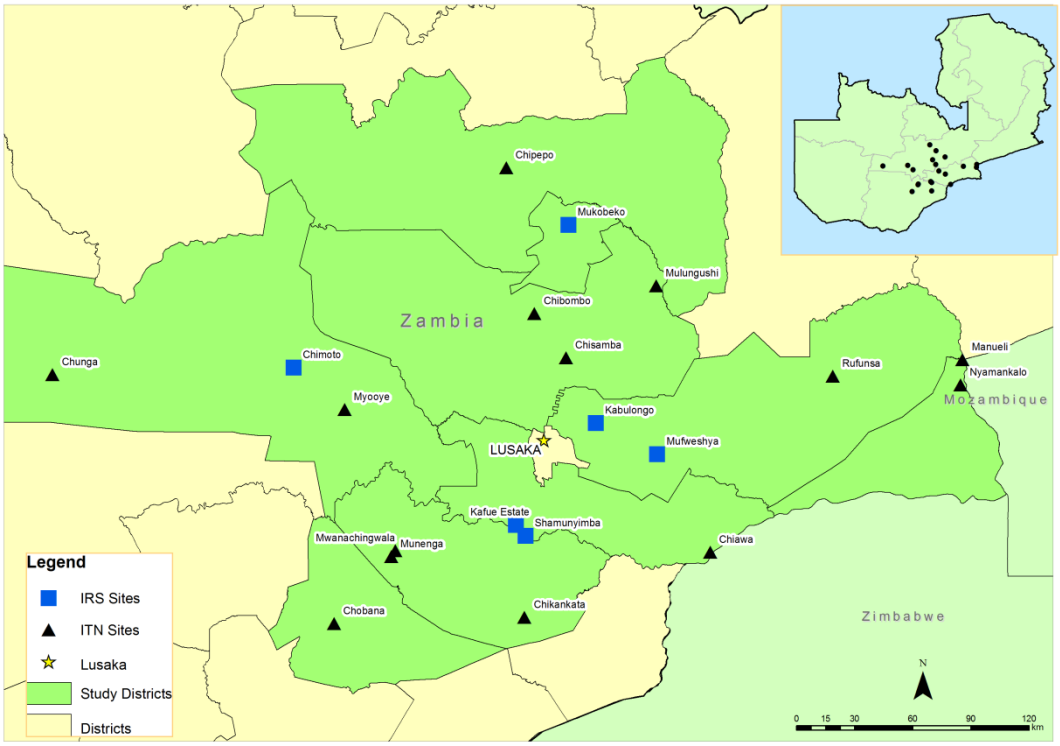


Figure 4.1: Map showing the spatial distribution of sentinel sites in Zambia

The mosquito collections were conducted in 5 sentinel sites where indoor residual spraying (IRS) was a predominant vector control activity and in 13 sites where insecticide treated bed nets (ITNs) are the predominant vector control tools (Figure 4.1).

An. gambaie s.s. was only detected in three sites, Chipepo, Manueli and Nyamankalo, whereas *An. arabiensis* was detected at thirteen sites, Chiawa, Chikankata, Chibombo, Chobana, Chipepo, Kabulongo, Manueli, Mukobeko, Mulungushi, Munenga, Nyamankalo, Rufunsa, and Shyamunyimba/Kafue estates. *An. funestus s.s* was detected at six sites, Chiawa, Chibombo, Kabulongo, Manueli, Mukobeko, and Nyamankalo.

In addition, a newly identified and provisionally named *An. funestus*-like species within the *An. funestus* species complex (Gillies and Coetzee 1987, Gillies and De Meillon 1968) was detected at four sites, Chibombo, Chipepo, Mukobeko and Nyamankalo. *Anopheles (Cellia) nili* Theobald, a member of the *An. nili* species group (Gillies and De Meillon 1968, Kengne et al. 2003) was identified from one site, Manueli.

4.2.2 Mosquito Abundance, Sporozoite Rates and Transmission Index

The calculated number of *An. gambiae s.s, An. arabiensis* and *An. funestus s.s* caught per window trap per 100 nights between April 2008 (04/08) and April 2009 (04/09) was 0.03, 1.59 and 0.12 respectively in IRS and ITN areas combined. For the subsequent period from May 2009 (05/09) to May 2010 (05/10) these values did not alter significantly 0.03, 1.21 and 0.08, respectively ($p>0.05$) (Table 4.1).

When ITN sites alone where considered for the period between 04/08 and 04/09, the calculated number of *An. gambiae s.s, An. arabiensis* and *An. funestus s.s* caught per window trap per 100 nights was 0.03, 1.46 and 0.12 respectively. In comparison, there was no significant difference with the values 0.03, 1.17 and 0.07 respectively from the period from 05/09 to 05/2010 ($p>0.05$) (Table 4.1 and Figure 4.2). The data from IRS sites also showed no significant difference in the calculated numbers of mosquitoes caught per window trap per 100 nights between the same two periods

0.00, 0.13 and 0.00, and 0.00, 0.04 and 0.01 respectively ($p>0.05$) (Table 4.1 and Figure 4.3). In comparing between ITNs and IRS areas, there was no significant change in the numbers caught in both periods ($p>0.05$).

However, if analysis is restricted to the main malaria transmission season of October to April, there is a statistically insignificant reduction in the number of *An. arabiensis* 2.14 (10/08-04/09) to 0.91 (10/09-4/10) and a small reduction in *An. funestus s.s* 0.16 to 0.05 ($P<0.05$). Note, no *An. gambiae s.s* were collected in this time period. Overall, no significant difference was observed between the two periods ($P>0.05$).

If only ITN sites are considered during the high malaria transmission season, there is a marked reduction of *An. arabiensis* 2.11 to 0.18 and a statistically insignificant small reduction in *An. funestus s.s* 0.16 to 0.05 caught per window trap per 100 nights ($p>0.05$). In the IRS areas, there was a small increase of *An. arabiensis* 0.03 to 0.10 during the same periods, although not statistically significant ($p>0.05$). However, no *An. funestus* were trapped during the peak transmission in IRS sites. Overall, there was no significant change in the numbers caught between the ITN and IRS areas in both periods 10/08-04/09 ($p>0.05$) and 10/09-4/10 ($p>0.05$) respectively.

In comparing ITN and IRS interventions over this period there was a bigger impact of the interventions on *An. gambiae s.s* and *An. funestus*, compared to *An. arabiensis*. The ITNs reduced the calculated number of *An. arabiensis* caught per window trap per 100 nights from a relatively low number to a minimum, but IRS brought them to below detectable levels.

Figure 4.2: Average number of *An. gambiae s.s, An. arabiensis* and *An. funestu s.s* per window trap per 100 nights, all ITN sites combined

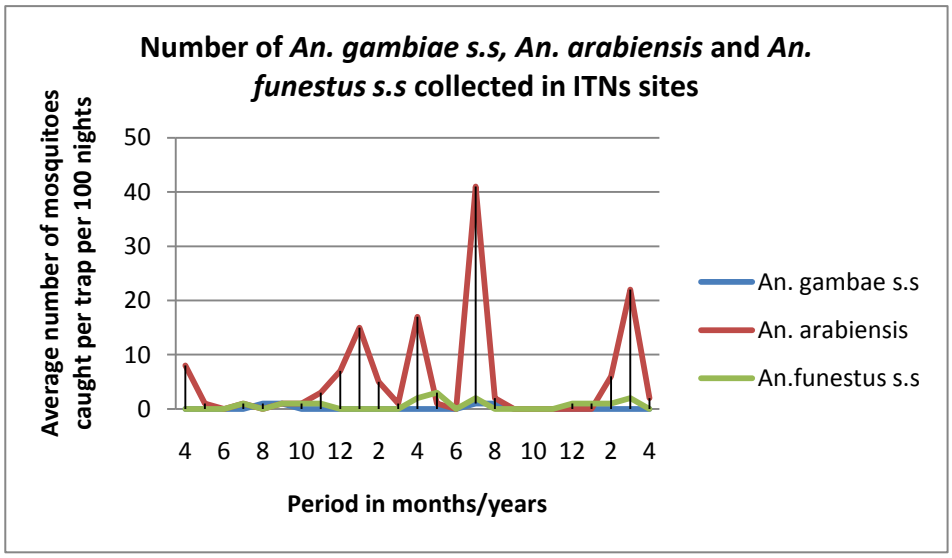
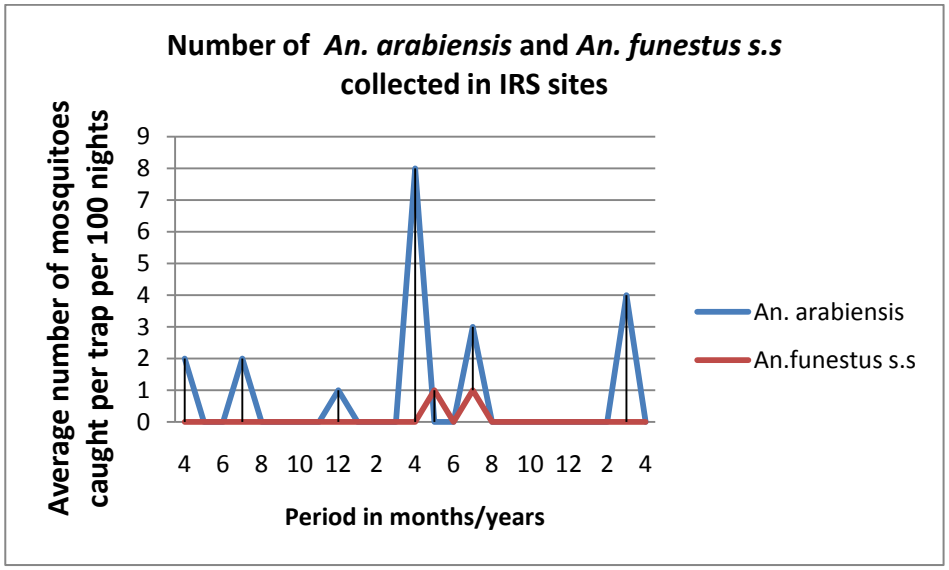


Figure 4.2 shows the number of mosquitoes caught from ITN sites between April 2008 and May 2010 ranging from < 1 per trap per 100 nights to 40 per trap per 100 nights for *An. gambiae ss and An. arabiensis* and *An. funestus s.l* respectively.

Figure 4.3: Average number of *An. arabiensis* and *An. funestus* s.s per window trap per 100 nights, all IRS sites combined.



As shown in figure 4.3, the number of mosquitoes caught from IRS sites between April 2008 and May 2010 ranged from < 1 per trap per 100 nights to 4 and 8 per trap per 100 nights for *An. arabiensis* and *An. funestus s.s* respectively.

Throughout this study none of 667 specimens, from the three major vectors, tested for infectivity were positive for *P. falciparum*. As such, the calculated transmission index was zero and therefore the transmission potential for all the three major malaria vectors: *An. gambiae s.s, An. arabiensis* and *An. funestus* was zero during the period of the study (Table 4.1) using this trapping method.

Culicine mosquitoes were collected, counted and recorded to ensure that in the absence of anophiline catches, the traps were being successfully operated. The numbers varied between sentinel sites, through-out the study period with densities from < 1 to 255.9 and from < 1 to 56.0 per trap per 100 nights in 2008 and 2010 respectively.

Table 4.1: Vector Abundance, Infectivity and Transmission index by period of time and intervention

	All sites		**All ITN sites**		**All IRS sites**	
Year	**04/08-4/09**	**05/09-5/10**	**04/08-4/09**	**05/09-5/10**	**04/08-4/09**	**05/09-5/10**
An. gambiae s.l						
No. caught	409	210	395	195	14	15
No. analyzed for species id	360	167	354	157	6	10
No. *An. gambiae* s.s	2	2	2	2	0	0
No. *An. arabiensis*	98	69	94	67	4	2
An. gambiae s.s propn (%)	0.49	0.95	0.51	1.03	0.00	0.00
An. gambiae s.s						
No. Estimated	2	2	2	2	0	0
No per trap per 100 nights	0.03	0.03	0.03	0.03	0.00	0.00
Sporozoite rate	0(n=2)	0(n=2)	0(n=2)	0(n=2)	0(n=0)	0(n=0)
Transmission index*	0	0	0	0	0	0
Transmission index$^{\infty}$	1	0	1	0	1	0
An. arabiensis						
No. Estimated	113	86	104	83	9	3
No per trap per 100 nights	1.59	1.21	1.46	1.17	0.13	0.04
Sporozoite rate	0(n=125)	0(n=98)	0(n=104)	0(n=92)	0(n=9)	0(n=6)
Transmission index*	0	0	0	0	0	0
Transmission index$^{\infty}$	1	0	1	0	1	0
An. funestus s.l						
No. caught	105	123	94	113	11	10
No. analyzed for species id	99	105	91	95	8	10
No. *An. funestus* s.s	8	5	8	4	0	1
An. funestus s.s propn (%)	7.62	4.07	8.51	3.54	0.00	10.00
An. funestus s.s						
No. Estimated	8	6	8	5	0	1
No per trap per 100 nights	0.12	0.08	0.12	0.07	0.00	0.01
Sporozoite rate	0(n=8)	0(n=9)	0(n=8)	0(n=6)	0(n=0)	0(n=1)
Transmission index*	0	0	0	0	0	0
Transmission index$^{\infty}$	1	0	1	0	1	0

* Number of infective mosquitoes per trap per 100 nights: $^{\infty}$Relative to baseline: Propn – proportion: id - identification

Table 4.2: Vector Abundance, Infectivity and Transmission index by period of time and intervention

	October to April All sites		October to April ITN sites		October to April IRS sites	
Year	**10/08-4/09**	**10/09-4/10**	**10/08-4/09**	**10/09-4/10**	**10/08-4/09**	**10/09-4/10**
An. gambiae s.l						
No. caught	187	38	186	31	1	7
No. analyzed for species id	187	38	186	31	1	7
No. *An. gambiae* s.s	0	0	0	0	0	0
No. *An. arabiensis*	82	35	81	31	1	4
An. gambiae s.s propn (%)	0	0	0	0	0	0
An. gambiae s.s						
No. Estimated	0	0	0	0	0	0
No per trap per 100 nights	0	0	0	0	0	0
Sporozoite rate	0(n=0)	0(n=0)	0(n=0)	0(n=0)	0(n=0)	0(n=0)
Transmission index*	0	0	0	0	0	0
Transmission index$^{\infty}$	1	0	1	0	1	0
An. arabiensis						
No. Estimated	82	35	81	31	1	4
No per trap per 100 nights	2.14	0.91	2.11	0.81	0.03	0.10
Sporozoite rate	0(n=82)	0 (n=35)	0(n=81)	0 (n=31)	0(n=1)	0 (n=4)
Transmission index*	0	0	0	0	0	0
Transmission index$^{\infty}$	1	0	1	0	1	0
An. funestus s.l						
No. caught	74	38	69	38	5	0
No. analyzed for species id	74	38	69	38	5	0
No. *An. funestus* s.s	6	2	6	2	0	0
An. funestus s.s propn (%)	8.11	5.26	8.70	5.26	0.00	0.00
An. funestus s.s						
No. Estimated	6	2	6	2	0	0
No per trap per 100 nights	0.16	0.05	0.16	0.05	0.00	0.00
Sporozoite rate	0(n=6)	0(n=2)	0(n=6)	0(n=2)	0(n=0)	0(n=0)
Transmission index*	0	0	0	0	0	0
Transmission index$^{\infty}$	1	0	1	0	1	0

* Number of infective mosquitoes per trap per 100 nights: $^{\infty}$Relative to baseline: Propn – proportion: id - identification

4.3 Discussion

Major malaria vectors occur sympatrically across Africa with variations in malaria transmission significance depending on behaviour, seasonal preferences and vectorial capacity (Gillies and Coetzee 1987, Bruce-Chwatt 1985, Coluzzi 1984, Fontenille and Simard 2004). Sound knowledge of their distribution is essential in guiding implementation of appropriate vector control interventions (Okara et al. 2010). In Zambia *An. gambiae s.s, An. arabiensis and An. funestus* are the principle malaria vectors in the country (DeMeillon 1937, Adams 1940, Watson 1953, Pielou 1947, Paterson 1963, Shelly 1973, Bransby-Williams 1979). The present findings corroborate these studies although the additional Afro tropical vectors of malaria,

An. nili, was also identified as was the recently described *An. funestus-like* species, whose role if any, in transmission has yet to be determined.

An. nili, an anthropophagic, endophagic but exophilic species that breeds in streams and rivers is a major vector in West Africa and a secondary vector in Central Africa (Krafsur 1970). This species was collected at Manueli sentinel site in Luangwa district at the end of the rainy season (April 2009) when the Luangwa River was at its highest level. Virtually all available data concerns West and Central African forms and *An. nili* from other regions remain poorly understood (Ndo et al. 2010, Krafsur 1970). This initial identification of *An. nili* in Zambia warrants the need for further entomological studies to ascertain the abundance and role this species may have in malaria transmission in the country.

An. funestus s.l specimens were identified by the method of Koekemoer et al. (2002) and the description of *An. funestus-like* distinct species within the *An. funestus* group (Spillings et al. 2009) increase our knowledge of the distribution range of this species which was first identified in Malawi. The involvement of this species in malaria transmission remains to be ascertained.

In this study, *An. gambiae s.s*, *An. arabiensis* and the zoophilic *An. quadriannulatus* where the only species identified within the *Anopheles gambiae* complex, with five species; *An. funestus s.s*, *An. parensis*, *An. rivulorum*, *An. leesoni*, *An. vaneedeni* and *An.funestus*-like identified as belonging to the *Anopheles funestus* group. However, only 4 *An. gambiae s.s,* 18 *An. funestus s.s* and 154 *An. arabiensis* were identified from all the eighteen sentinel sites during the entire study period compared to the 176 *An. gambiae s.s,* 36 *An. funestus* and 111 *An. arabiensis* collected from three low transmission districts, Chibombo, Ndola and Chingola (Siachinji et al. 2001), and the 292 *An. gambiae s.s,* 815, *An. funestus* and 148 *An. arabiensis* collected in a high transmission area of Mwense district using exit window traps (Chimumbwa 2003) over one year before the scaling up of interventions (Table 4.3). This decline in vector abundance and infectivity demonstrates the impact that effective and consistent interventions have had on mosquito populations.

Table 4.3: Pre-vector control intervention indoor resting malaria vector collection abundance and sporozoite rates

Reference	Site	*Ecotype*	Abundance of indoor resting malaria vectors			Sporozoite rates of indoor resting malaria vectors			
			An. gambiae s.s	*An. arabiensis*	*An. funestus*	*An. gambiae s l*	*An. arabiensis*	*An. gambiae s.s*	*An. funestus*
Paterson, 1963	Chirundu	Hot riverine valleys	-	-	-	2.3	-	-	-
Zahar, 1985	Chirundu	Hot riverine valleys	-	-	-	3	-	-	0
	Ndola	Savanna plateaus	-	-	-	1.6	-	-	1.6
	Livingstone	Hot riverine valleys	-	-	-	2.4	0.18	-	-
Shelly, 1973	Chirundu	Hot riverine valleys	-	-	-	1.2	-	-	-
Bransby-Williams, 1979	Chipata	Savanna plateaus	-	981	-	-	1.1	-	-
	Lusaka	Savanna plateaus	-	-	-	-	0	-	-
Chimumbwa, 2003	Lukwesa	Luapula river valley	271	29	648	-	0	5.9	4.4
	Kapululila	Hot riverine valleys	21	119	167	-	5.6	0	0
Siachinji et al, 2001	Chibombo	Savanna plateaus	29	115	13	-	-	-	-
	Ndola	Savanna plateaus	127	5	23	-	-	-	-
	Chingola	Savanna plateaus	20	0	0	-	-	-	-
Siachinji et al, 2002	Macha	Savanna plateaus	-	-	-	-	4.23	-	-
Kent et al, 2007	Chidakwa	Savanna plateaus	-	-	-	-	1.6	-	-
	Lupata	Savanna plateaus	-	-	-	-	18.3	-	-

The detection of *An. parensis*, *An. rivulorum. An. vaneedeni* and *An. leesoni* validates the findings of Siachinji et al. (2001) who identified these species in Chibombo and Ndola. While *An. rivulorum* and *An. leesoni* have a wide geographical distribution throughout Africa and are sympatric with other species in the group, the former has also been identified in Tanzania as a local vector (Wilkes et al. 1996). Therefore, the identification of *An. rivulorum* and *An. nili* could have implications for malaria transmission in Zambia.

An. vaneedeni was thought to have a confined geographical distribution in Mpumalanga and Kwazulu Natal provinces in South Africa and *An. parensis* confined to East Africa (Kenya and Tanzania) and Kwazulu Natal in South Africa (Gillies and Coetzee 1987, Gillies and DeMeillon 1968). Detecting both these species in Zambia has increased our knowledge of the geographical distribution of these zoophilic members of the *An. funestus* group. Notably, results from the few entomological studies in Zambia (Kent et al. 2007, Kent et al. 2007, Siachinji et al. 2001, Siachinji and Mulenga 2002, Chimumbwa 2003, DeMeillon 1937, Adams 1940, Watson 1953, Pielou 1947, Paterson 1963, Shelly 1973, Bransby-Williams 1979) exhibit great heterogeneity in species composition and their relative abundance throughout the country.

The impact of malaria control interventions can be evaluated through several methods including repeated population-based surveys; parasite prevalence, malaria–specific mortality and all course mortality. While prevalence of parasites in children has been frequently used as a proxy measure for malaria transmission intensity, regardless of the non-linear relationship between prevalence and transmission intensity, as measured by the entomological inoculation rate (EIR) (Beier et al. 1999), the potential of routine entomological surveillance data including vector abundance, infectivity and insecticide resistance in evaluation studies have not been fully exploited (WHO 2009).

Overall results on the impact of interventions on the vector abundance showed no appreciable variation in the number of *An. gambiae* s.s, *An. arabiensis* and *An. funestus* trapped between April 2008 and April 2009 in comparison to the period of May 2009 to May 2010 (Table 4.1, Table 4.2) ($p>0.05$). The numbers of *An.*

arabiensis were relatively higher than those of *An. gambiae* s.s and *An. funestus* in both periods. This trend was consistent with the results from the ITN deploying sites ($p>0.05$). The notable increase in the number of *An. arabiensis* is likely to have been due to the above average rains received in the country in 2009 relative to 2008 and 2010 rainy seasons. However, in IRS implementing localities an exceptionally sharp decline in the number of *An. arabiensis* and *An. funestus* was demonstrated, with the total absence of *An. gambiae* s.s (Table 4.1 and Figure 4.3).

The relative abundance of house exiting *An. gambiae* s.s, *An. arabiensis* and *An. funestus s.s* during the peak malaria transmission season (October to April) also showed marked heterogeneity in this study (Table 4.2). There were no *An. gambiae s.s* trapped during this period, and combined results from all sites showed a marked decline of *An. arabiensis* and a small reduction in *An. funestus s.s* exiting houses the period from October 2008 to April 2009 compared to the period from October 2008 to April 2009. In the ITNs there was a significant reduction of *An. arabiensis* and a slight decline in *An. funestus s.s* ($P>0.05$). Overall, the biggest impact of the two interventions was on *An. gambiae* s.s, and *An. funestus s.s* compared to *An. arabiensis.* The ITNs reduced the calculated number of *An. arabiensis* to a minimum, but IRS brought them to below detectable levels (Figures 4.2 and 4.3). The end of the rainy season coincides with the peak in abundance of the three major vectors (Rogers et al. 2002, Gillies and De Meillon 1968, Smith et al. 1993). In this study, the estimated numbers of *An. arabiensis* also peaked during this period. However, the relative abundance of the house exiting *An. gambiae* s.s, *An. arabiensis* and *An. funestus* s.s was reduced in IRS areas relative to ITN areas.

Throughout this study, which started after five rounds of IRS and seven years of ITN delivery, none of the trapped mosquitoes tested for infectivity was positive for *P. falciparum* sporozoites. As such, the transmission potential for all the three major vectors of malaria: *An. gambiae s.s*, *An. arabiensis* and *An. funestus* was zero as expressed by the calculated transmission index following the effective and consistent implementation of interventions in operational areas (Table 4.1 and 4.2). In addition to the demonstrated impact of IRS and ITNs, the lack of sporozoites and transmission potential can also be ascribed to the low numbers of mosquitoes caught due to flaws in the exit window trap method and a change in the population structure

of the vectors, particularly in relative densities of *An. arabiensis* following deployment of interventions, coupled to the effective case management using Coartem® (ACTs) and the improved health care seeking behaviour of residents. There was also a lack of stock outs of ACTs at health facilities during this period.

While the malaria transmission efficiency of vectors and their amenability to control interventions vary markedly (Bruce-Chwatt 1985), by monitoring species density and infectivity it is possible to measure the direct effect that the vector control programme is having on transmission of malaria (Sharp *et al* 2007). To this effect, Protopopoff et al. (2007) and Sharp et al. (2007) have demonstrated that in the presence of *An. funestus* and *An. gambiae s.s* both IRS and ITN may need to be combined to effectively reduce the densities and sporozoite rates of these indoor resting species. The present findings show complete elimination of *An. gambiae s.s* from operational settings of these interventions and a marked suppression of *An. funestus s.s* throughout the study period, albeit with an increase in *An. arabiensis* in July and August of 2009 and March of 2010. These temporal results further validate the findings by Lengeler and Sharp (2003) that *An. gambiae s.s and An. funestus* are characteristically more amenable to control by IRS and ITNs than *An. arabiensis.* However, the predominance of *An. arabiensis* after the effective deployment of interventions may be attributed to its exophilic nature and its catholic feeding behavior, thus rendering it evasive to the effects of indoor targeted control interventions.

Anopheles arabiensis predominated in four sites; Chiawa, Munenga and Nyamankalo that are in the low rainfall southern zone and in Luangwa that also exhibited the highest densities of *An. funestus s.s* and *An. gambiae s.s* due to the year round presence of breeding sites. *An. funestus-like* species was detected in sites in the central low rainfall zone, with low numbers of *An. arabiensis* with *An. funestus s.s* being present. The predominance of *An. arabiensis,* a vector associated with unstable malaria transmission (Fonteinille and Lochouarn, 1999), in most sites implies that it may be contributing to the perpetuation of malaria transmission in the country, as demonstrated by the earlier studies (Shelly 1973, Bransby-Williams 1979, Zahar 1985).

The predominance of *An. arabiensis*, a more exophilic and exophagic species, in vector control operational settings necessitate scaled up implementation of Larval Source Management strategies (environmental management and larviciding) to facilitate the complete control of this behaviourally facultative malaria vector. The continued presence of both *An. arabiensis* and *An. funestus* in intervention areas may have implications of possible failure for the malaria control programme. It may also indicate that insecticide resistance could have been selected within the populations of these vectors, thus making resistance surveillance imperative for the malaria control programme.

The present intensive malaria vector control efforts in Zambia have resulted in marked changes in the abundance of *An. funestus s.s* and *An. gambiae s.s* in operational settings for IRS and ITNs as demonstrated in monitoring sentinel sites (Figure 4.1.). *Anopheles funestus* was identified only in 6 of the eighteen sentinel sites and was predominantly found in sites with ITNs alone (Chiawa, Chibombo, Manueli and Nyamankalo) than those with IRS (Kabulongo and Mukobeko). The only four *An. gambiae s.s* were identified from two ITN sites (Chipepo and Nyamankalo), and one IRS area (Manueli). *Anopheles arabiensis* was identified in 13 sentinel sites and occurred predominantly in ten ITN sites (Chiawa, Chikankata, Chibombo, Chobana, Chipepo, Manueli, Mulungushi, Munenga, Nyamankalo, and Rufunsa) and in only three sites with IRS (Kabulongo, Mukobeko and Shyamunyimba). These findings demonstrate that in addition to markedly reducing mosquito densities and eliminating infectivity, vector control has resulted in a shift in species composition, as reported previously (Shelly 1973, Bransby-Williams 1979, Lindsay et al. 1998). This could also explain the low transmission levels (meso-to hypo-endemicity) of malaria in these areas and further validates the assumption that IRS has a more prompt and powerful impact than ITNs.

Several studies on comparative operational impact of IRS and ITNs upon malaria transmission have been conducted (Neville et al. 1996) and both interventions have been found to be effective in a large number of epidemiological settings (Lengeler and Sharp 2003). Though IRS with DDT eliminated *An. funestus* from operational areas without pyrethroid-based resistance (Maharaj et al. 2005), the results from this study indicate the complete elimination of *An. gambiae* s.s from IRS sites and

suppression of *An. funestus* and *An. arabiensis* to a minimal level, coupled to the absence of vector infectivity in both IRS and ITNs settings. Hence the two interventions are not mutually exclusive (Guessan et al. 2007) as they both protect all individuals within a community by reducing densities and infectivity of malaria vectors and thus overall transmission (Lengeler 2004, Killeen et al. 2006).

This study also demonstrates that at low vector densities exit window traps are not particularly effective for monitoring the impact of these interventions, as indicated by the low numbers of collections per trap per night across the study period. This situation reduces the possibility of collecting infected mosquitoes at the monitoring sites. The fact that *An. arabiensis* predominates in collections from most sites further compromises the efficacy of these traps, especially in sites where animal husbandry is predominant. The performance and efficiency of the traps was also compromised by the lack of compliance by householders, particularly in rural sentinel sites. Several factors contributed to this: The high turn-over of individuals trained to empty the traps during the cultivation, weeding and harvesting periods of the year; this was also a factor in sites located in areas where fishing was the main livelihood activity; traditional practices of abandoning a house after the head of the house passes on, including the myths of associating the black cloth on the trap with Satanism and Witchcraft affected the acceptance of the traps by potentially literate householders. More importantly, often no mosquitoes are trapped in areas of low mosquito numbers leading to non-compliance.

Though *An. nili* and the *An. funestus-like* species have not been implicated in malaria transmission in this study, their presence underscores the influence of local ecology on malaria transmission and unveils the great diversity of the malaria vectorial system in the country that should be taken into account in malaria vector control policy decision making. Further insight into the transmission potential and population structure of these species will be exceptionally useful in the development of locally-adapted vector control measures.

The impact of intensive large scale insecticide based vector control using IRS and ITNs has been demonstrated by the dramatic elimination of *An. gambiae s.s* from operational settings and suppression of both *An. arabiensis* and *An. funestus*

densities to minimal levels coupled with the absence of sporozoites and thus creating a void in their transmission potential as expressed by the transmission index.

While this study has shown that entomological monitoring and evaluation is an indispensable tool for rational large scale malaria vector control using IRS and ITNs, it has shown that progress and efficiency of exit window traps in low transmission zones is compromised by the non-compliance of householders. Therefore, monitoring of indoor vector densities should be streamlined by replacing or complimenting the exit window traps with a more robust collection tool like the CDC light trap coupled with the involvement of dedicated technical staff for close monitoring of their operations.

The recent shift in strategic emphasis from malaria control to elimination and eradication has highlighted major gaps in knowledge that need to be addressed before such achievement is contemplated (Feachem and Sabot 2008, Feachem et al. 2009, Mendis et al. 2009). While basic knowledge in vector biology, ecology and genetics is well understood, there is need to integrate these entomological parameters into routine surveillance systems. This study was conducted in low transmission settings achieved primarily by successful malaria vector control. The fact that transmission index is below 1 (Tables 4.1 and 4.2) means that the disease will keep reducing. However, any strategy that targets reduction of transmission down to the level where elimination is within reach will need to strengthen its surveillance systems through very effective malaria decision support systems.

CHAPTER FIVE

Monitoring the operational impact of vector control on insecticide resistance profiles of major malaria vectors in Zambia

5.1 Introduction

In the absence of a vaccine, malaria control programmes rely on the combination of effective vector control and efficacious treatment of clinical cases. Indoor Residual Spraying and ITNs are the most common form of vector control, both of which have been shown to be successful in controlling malaria vectors (Neville et al. 1996, Lengeler and Sharp 2003, Lengeler 2004). Both IRS and ITNs rely on the use of insecticides. There are only 12 registered insecticides for IRS from four classes and 6 for ITNs all from the same class, pyrethroids (WHOPES a 2007, WHOPES b 2007).

Extensive exposure of vectors to insecticides can often select for insecticide resistance (Collins et al. 2000, Hemingway and Ranson 2000, Coleman and Hemingway 2007) and the emergence of resistance in *Anopheles* species in Africa is a major concern for the successful and sustainable implementation of insecticide-based malaria control programmes (Hargreaves et al. 2000).

Knowledge of the basic mechanisms of insecticide resistance and factors contributing to its emergence, its extent and the distribution of resistant populations are well established (Hemingway and Ranson 2000, Coetzee 2004, Hemingway and Bates 2003, Grant and Matsumura 1989, Hemingway et al. 1985, Brown 1986, Brogdon and McAllister 1988). Selection of resistance in vector populations is dependant both on the volume and frequency of applications of insecticides used against them and inherent characteristics of the insect species involved (Collins et al. 2000).

DDT was first introduced for malaria control in 1944 (Hays 2000, Giglioli et al. 1974, Gabaldon 1983) and was the main insecticide used in the WHO-led malaria eradication campaign between 1955 and 1969 in combination with treatment of the disease using chloroquine and quinine (Najera 1989). Emerging insecticide resistance alongside drug resistance, are considered major reasons for the failure of the eradication campaign (Trigg and Kondrachine 1998). Today resistance to all classes of insecticides has been detected in the three main African malaria vectors: *Anopheles gambiae s.s*, *An. arabiensis* and *An. funestus s.s.* in different parts of

Africa (Coetzee 2004, Coleman et al. 2006).

The development of pyrethroid resistance in *An. gambiae s.l* and *An. funestus* is particularly important given the emphasis by the WHO and other organizations on the use of pyrethroid impregnated bed nets for malaria control (Chandre et al. 1999, Vulule et al. 1994, Soderlund and Bloomquist 1989). While insecticide resistance is not a new phenomenon, with DDT resistance initially documented in 1956 (WHO, 1957) just 11 years after its introduction (Mabaso et al, 2004), the documentation and understanding of insecticide resistance in malaria vectors in Zambia is minimal. With the reinvigoration of vector control efforts primarily based on the use of IRS and ITNs in Zambia, following the recent boost in funding for malaria control (Komatsu et al. 2007), the risk of emergence of *Anopheles* species resistant to insecticides widely used for vector control is likely to be exacerbated. This will threaten long-term ability to control malaria, which is endemic country-wide, particularly as resistance is evolving at a faster rate than new insecticides are being developed and marketed (Hemingway et al. 2006, Coleman et al. 2006).

If not monitored directly, resistance will only be detected once operationally significant increases in disease transmission and childhood mortality occur. However, the historical response of waiting until an epidemiologically significant endpoint for disease, to assess whether an insecticide has failed, is no longer sustainable. Resistance management is essential if the scarce public health resources are to be conserved (Coleman et al. 2006).

There is increased evidence for the selection of knock down resistance (*kdr*) alleles associated with the massive deployment of ITNs and IRS (Protopopoff et al. 2007, Diabate et al. 2006, Dabire et al. 2006). Another compounding factor is the association of resistance in the *Anopheles* species with the agricultural use of insecticides (Mouchet 1988, Diabate et al. 2002). Selection for resistance in mosquitoes by agricultural use of insecticides is well documented, and can severely compromise vector control (Mouchet 1988, Roberts and Andre 1994). It is therefore essential to know where the selective pressure on *Anopheles* comes from, to facilitate viable insecticide resistance management.

To prolong the effectiveness of the currently available insecticides and thereby prevent control failure, it is vital to detect the emergence of resistance at an early stage, so that appropriate action can be taken. Detection of insecticide resistance, accompanied by biochemical and molecular assays to identify the underlying resistance mechanisms are essential (Hemingway et al. 1997, Penilla et al. 1998, Wondji et al. 2002, Collins et al. 2000). This information can then be used for rational resistance management, with a view to controlling the development and spread of resistant vector populations (Hemingway and Ranson 2000).

The National Malaria Control Program (NMCP) in Zambia has a successful history of insecticide use for the control of the malaria vectors *An. funestus*, *An. gambiae* s.s. and *An. arabiensis*. DDT was sprayed in Zambia from 1947 to 1980 with no obvious manifestation of DDT resistance in the vectors, either measured directly from sporadic bioassays or implied from increases in malaria transmission. Indoor residual spraying with DDT and pyrethroids was reintroduced in 2000 by the private sector (Sharp et al. 2002). This intervention, alongside ITN distribution was incrementally implemented as the major vector control intervention from 2003. Pyrethroids remain the only insecticides currently available for use on bed nets, and there are also restrictions on the number of insecticides suitable for IRS coupled to constraints that may be imposed on insecticide choice by the insecticide resistance profile of the targeted mosquito vector population and the registration of insecticides within Zambia.

To ensure that insecticide choice for the IRS program is effective and evidence-based, insecticide resistance surveillance and assessment of potential resistance mechanisms within the targeted vector populations is essential to an insecticide based vector control programme.

This work reports on the insecticide resistance profiles of major malaria vectors from Zambia with the view of informing insecticide-use policy formulation.

5.2 Results

5.2.1 Mosquito Collections.

Mosquitoes were collected from 17 localities, 11 of which were sentinel sites, from 10 districts in Zambia (Figures. 5.1 and 5.2). A total of 1,742 *An. gambiae s.l* and 796 *An. funestus s.l,* 1-3 day old F1 mosquitoes reared from 52 and 28 wild caught females respectively, were assayed for insecticide susceptibility using the WHO protocol (W.H.O 1998).

5.2.2 Susceptibility Assays.

WHO insecticide resistance assay results were categorised according to percentage mortality (Table 5.1) as susceptible, requiring confirmation of resistance, or resistant. This standard is recommended by WHO (WHO. 2005) and has been used by the African Network for Vector Resistance (ANVR) and has been adopted for this thesis (Table 5.1).

Table 5.1: Criteria for interpretation and classification of results, based on WHO recommendations:

	At least 80 mosquitoes tested per bioassay	Twenty to 79 mosquitoes tested per bioassay
Susceptible	Mortality 98 – 100 %	Mortality 98 – 100 %
Resistance suspected, to be confirmed	Morality 95 – 97 %	Mortality 80 – 97 %
Resistance	Mortality < 95 %	Mortality < 80 %

DDT and pyrethroids where prioritized for testing because they are currently in use for malaria vector control in Zambia. Only a few tests were conducted with carbamate and organophosphate insecticides.

Prior to 2009, no resistance to the pyrethroids or DDT had been detected in *An. gambiae s.l.* in Zambia (Table 5.2 and figure 5.1). Between 2009 and 2010 resistance to the pyrethroid deltamethrin was detected in 7 localities, Chipepo (41.8%), Chipulukusu (13.5%), Kizingezinge (95.2%), Mushili (41.0%),

Mwanachingwala (75.0%) Myooye (93.2%) and Nyamankalo (90.9%). Both Chipulukusu and Mushili also showed resistance to permethrin 61.0% and 55.0% respectively and resistance was also detected to lambda cyhalothrin at Nyamankalo (83.3%) although the sample size was small (n=6). Complete susceptibility was detected to the pyrethroid deltamethrin at Chiawa (100%), Nanga Farms (100%) and Mukobeko (100%) and to permethrin in Nyamankalo (100%) (Table 5.2 and figure 5.1).

Resistance to DDT was detected at Chipulukusu (43.0%), Kizingezinge (3.8%), Mushili (11.0%) and Myooye (69.0%). The rapid selection of both pyrethroid and DDT resistance suggests potential *kdr* resistance. Complete susceptibility to DDT was detected at Kafue, Nanga farms and Nyamankalo, but sample sizes did not exceed 8 mosquitoes (Table 5.3). Only one site Mushili was tested for malathion resistance and one site Nyamankalo was tested for the carbamate bendiocarb, both of which were fully susceptible.

When 2009 and 2010 data was compared with 1999 data for *An. gambiae s.l* for deltamethrine resistance in Mukobeko, no significant difference ($p > 0.1$) was observed by Chi-square test. A significant change in resistance levels were detected to permethrin and to DDT in Chipulukusu and Mushili respectively ($p < 0.001$) when compared to 1999 data, implying the selection of resistance during the IRS scale up campaign in these districts (Table 5.2).

Prior to 2009 no insecticide resistance was detected to pyrethroids or DDT in F1 and F0 *An. funestus s.l.*. Between 2009 and 2010, resistance to the pyrethroid deltamethrin was detected in 9 localities, Chibombo (88.9%), Kabulongo (80.0%), Kafue (95.6%), Manueli (72.7%), Mukobeko (96.0%), Mwanachingwala (81.8%), Myooye (96.2%), Nyamankalo (80.5%), and Rufunsa (66.6%). Resistance was also detected to the pyrethroid permethrin at Nanga farms (90.9%) although the sample size was small (n=11). Complete susceptibility was detected to deltamethrin at Chipepo (100%), Mufweshya (100%) and Nanga farms (100%) and to lambda-cyhalothrin at Mulungushi (100%) (Table 5.3 and figure 5.2).

Resistance to DDT was detected at Kafue (98.0%) and Myooye (94.0%). Complete susceptibility to DDT was detected at Kabulongo, Katete, Mufweshya and Nanga farms but the sample sizes were low for Nanga farms and Katete, not exceeding 10 mosquitoes (Table 5.3 and figure 5.2). Only one site Mulungushi was tested for bendiocarb resistance and there was 100% susceptibility to this insecticide.

Comparing 2009/10 data for *An. funestus s.l* resistance to deltamethrin in Chibombo using the Chi-square statistic, a significant difference ($p < 0.001$) was observed relative to 1999. However, no significant difference in resistance was detected to the same insecticide in Mukobeko ($p > 0.1$) relative to 1999 (Table 5.3).

5.2.3 Knockdown Resistance (*kdr*)

Pyrethroid and DDT resistance were detected in the same population from Chipulukusu, Kafue, Mushili and Myooye which suggested the potential for cross-resistance conferred by the target site, *kdr*-type resistance (Martinez-Torres et al. 1998; Ranson et al. 2000). One hundred and Sixty-five survivors of DDT and pyrethroid exposure *An. gambiae s.s* were tested for both east (leu-ser) (Ranson et al. 2000) and west (leu-phe) (Martinez-Torres et al. 1998) *kdr*. All 165 *An. gambiae s.s* were identified as the molecular s-form and only the west (leu-phe) *kdr*-type mutation was detected in 155 samples (Table 5.4).

Table 5.2: WHO susceptibility test results on 1-3 dayold *An. gambiae s.l* of 17 localities in Zambia

Location	Data from 1999								Data 2009-2010											
	deltamethrin (0.05%)		permethrin (0.75%)		λ-cyhalothrin (0.05%)		DDT (4%)		deltamethrin (0.05%)		permethrin (0.75%)		λ-cyhalothrin (0.05%)		DDT (4%)		Malathion (5%)		Bendiocarb (0.01%)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Chiawa	-	-	-	-	-	-	-	-	3	100	-	-	-	-	-	-	-	-	-	-
Chibombo*	81	100	-	-	-	-	11	100	-	-	-	-	-	-	-	-	-	-	-	-
Chingola*	15	100	-	-	-	-	5	100	-	-	-	-	-	-	-	-	-	-	-	-
Chipepo	-	-	-	-	-	-	-		43	41.8	-	-	-	-	-	-	-	-	-	-
Chipulukusu*			46	100 [b]			121	100 [b]	96	13.5	19	61	-	-	428	43	-	-	-	-
Kafue	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	100	-	-	-	-
Kizingezinge	-	-	-	-	-	-	-	-	105	95.2	-	-	-	-	157	3.8	-	-	-	-
Livingstone[#]	17	100	-	-	13	100	32	100	-	-	-	-	-	-	-	-	-	-	-	-
Lusaka[#]	7	100	-	-			6	100	-	-	-	-	-	-	-	-	-	-	-	-
Mukobeko[#]	19	100 [a]	-	-	11	100	9	100	16	100	-	-	-	-	-	-	-	-	-	-
Mushili	-	-	-	-	-	-	73	100 [b]	60	41	31	55	-	-	100	11	47	100	-	-
Mwanachingwala	-	-	-	-	-	-	-	-	4	75	-	-	-	-	-	-	-	-	-	-
Myooye	-	-	-	-	-	-	-	-	74	93.2	-	-	-	-	73	69	-	-	-	-
Nanga Farms	-	-	-	-	-	-	-	-	8	100	-	-	-	-	5	100	-	-	-	-
Nyamankalo	-	-	-	-	-	-	-	-	11	90.9	4	100	6	83.3	8	100			10	100
Samfya[#]	8	100			5	100	7	100	-	-	-	-	-	-	-	-	-	-	-	-

% =percentage mortality [a]=p>0.1 [b]=p<0.001, * =Unpublished baseline data collected by TDRC, [#] Unpublished data collected by NMCP

Table 5.3: WHO susceptibility test results on 1-3-d-old *An. funestus s.l* of 17 localities in Zambia

Location	Data from 1999										Data 2009-2010											
	deltamethrin (0.05%)		λ-cyhalothrin (0.05%)		DDT (4%)		Propoxur (0.01%)		Malathion (5%)		deltamethrin (0.05%)		permethrin (0.75%)		λ-cyhalothrin (0.05%)		DDT (4%)		Malathion (5%)		Bendiocarb (0.01%)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Chibombo*	72	100 [b]	-	-	-	-	19	100	-	-	9	88.9	-	-	-	-	-	-	-	-	-	-
Chingola*	-	-	-	-	-	-	3	100	16	100	-	-	-	-	-	-	-	-	-	-	-	-
Chipepo	-	-	-	-	-	-	-	-	-	-	4	100	-	-	-	-	-	-	-	-	-	-
Kabulongo	-	-	-	-	-	-	-	-	-	-	15	80	-	-	-	-	14	100	-	-	-	-
Kafue	-	-	-	-	-	-	-	-	-	-	23	95.6	-	-	-	-	90	98	-	-	-	-
Katete	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	100	-	-	-	-
Livingstone[#]	5	100	7	100	6	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Manueli	-	-	-	-	-	-	-	-	-	-	11	72.7	-	-	-	-	-	-	-	-	-	-
Mufweshya	-	-	-	-	-	-	-	-	-	-	18	100	-	-	-	-	21	100	-	-	-	-
Mukobeko[#]	25	100 [a]	15	100	25	100	-	-	-	-	26	96	-	-	-	-	-	-	-	-	-	-
Mulungushi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	100	-	-	-	-	7	100
Mushili*	4	100	-	-	-	-	-	-	4	100	-	-	-	-	-	-	-	-	-	-	-	-
Mwanachingwala	-	-	-	-	-	-	-	-	-	-	22	81.8	-	-	-	-	-	-	-	-	-	-
Myooye	-	-	-	-	-	-	-	-	-	-	27	96.2	-	-	-	-	62	94	--	-	-	-
Nanga Farms	-	-	-	-	-	-	-	-	-	-	30	100	11	90.9	-	-	10	100	-	-	-	-
Nyamankalo	-	-	-	-	-	-	-	-	-	-	87	80.5	-	-	-	-	33	88	-	-	-	-
Rufunsa	-	-	-	-	-	-	-	-	-	-	66	66.6	-	-	-	-	-	-	-	-	-	-

% =percentage mortality [a]=p>0.1 [b]=p<0.001, * =Unpublished baseline data collected by TDRC, [#] Unpublished data collected by NMCP

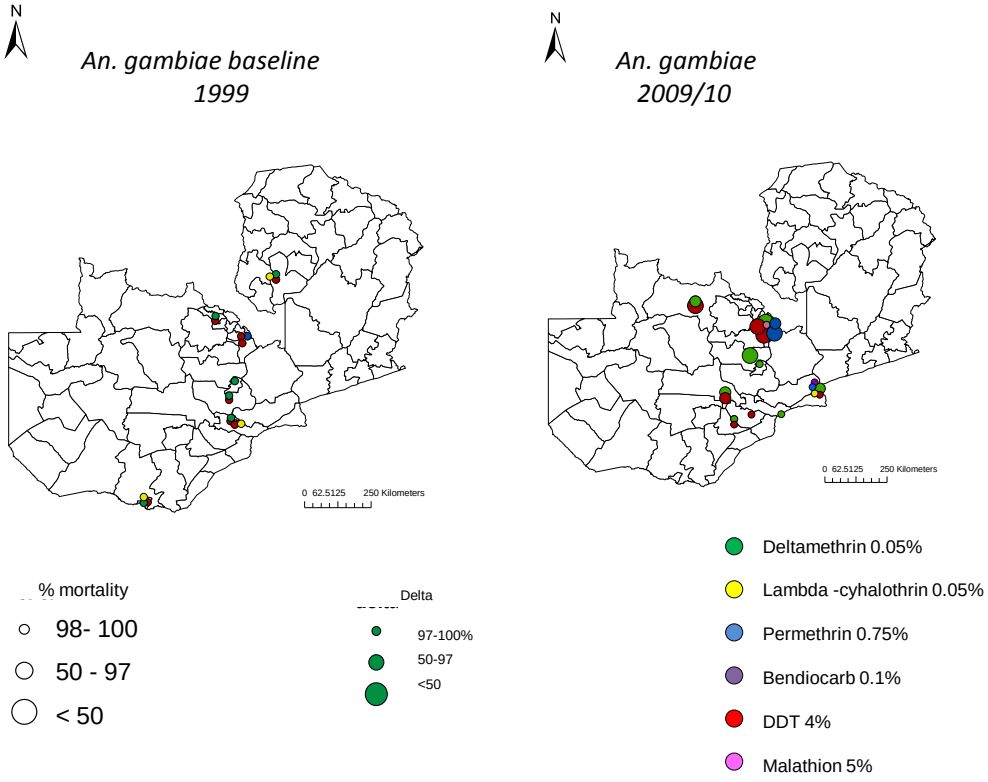


Figure 5.1: The spatial distribution of insecticide resistance in *An. gambiae s.l.* in 1999 compared to 2009/10 in Zambia.

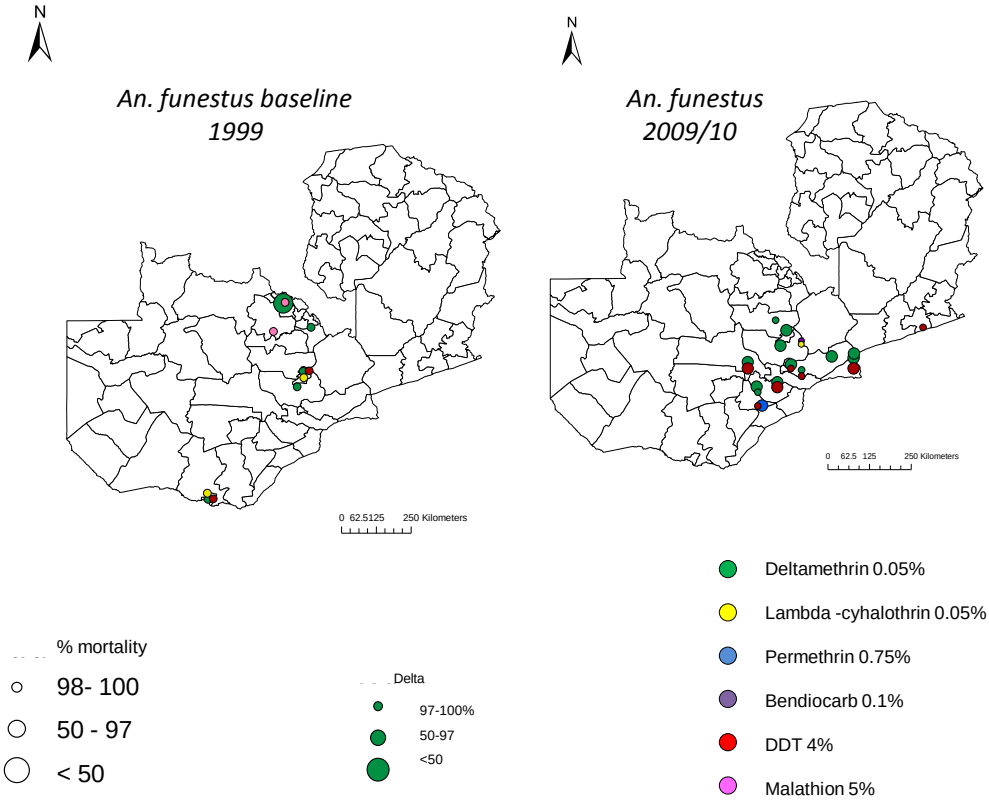


Figure 5.2: The spatial distribution of insecticide resistance in *An. funestus s.l* in 1999 compared to 2009/10 in Zambia.

Table 5.4: Knock down resistance (*kdr*) test results on 1-3 day old *An. gambiae s.s* from 6 localities in Zambia

Location	Homozygous Leu-Phe mutation	Heterozygous	Homozygous wild type
Chipepo	21	0	0
Chipulukusu	45	1	0
Kizhingezhinge	20	0	0
Mushili	58	3	0
Myooye	1	0	10
Twapya	6	0	0

5.3 Discussion

The selection of insecticide resistance has great potential to compromise any insecticide-based malaria vector control programme (Hemingway et al. 1997, Collins et al. 2000). The number of insecticides and formulations recommended by the WHO Pesticide Evaluation Scheme (WHOPES) for IRS and ITNs is severely limited (WHO 2001). This arsenal may be further depleted by the lack of good stewardship of available public health insecticides (Coleman and Hemingway 2007). As such, country-specific and regional insecticide registration and regulation is imperative, coupled with active monitoring and management of resistance levels in field populations.

In Zambia, intensive malaria vector control using indoor residual spraying with DDT and pyrethroids and community-based distribution of insecticide treated bed nets has been implemented since 2000. Early data from 1999 showed no resistance in *An. gambiae* s.s., *An. arabiensis and An. funestus* to these and other insecticides. Excessive insecticide utilisation has exacerbated selection for insecticide resistance among the vectors they are intended to control (Hemingway and Bates 2003). To ensure that the insecticides used for IRS in Zambia remain effective and their choice is evidence-based, a malaria decision support system incorporating insecticide resistance surveillance was established in Zambia and the resistance profile was monitored in seventeen localities using standard WHO susceptibility bioassays.

In this study, the bioassay results using WHO discriminating dosages showed a high level resistance of both *An., gambiae s.l* and *An, funestus s.l* to pyrethroids and DDT

in Zambia, following 10 years of consistent vector control implementation. There is evidence of significant resistance of *An. gambiae s.l* to deltamethrin in both IRS and ITN areas, to permethrin in IRS areas, to lambda-cyhalothrin in ITN areas and to both DDT and deltamethrin in the IRS areas. Equally, high levels of resistance of *An. funestus s.l* to DDT in IRS areas as well as the low and high level resistance to deltamethrin in ITN areas was detected. Comparing areas under the two interventions, there is marked heterogeneity in the level of resistance between IRS and ITN sites over time. The levels of resistance in An. gambiae s.l in IRS areas were higher than those in ITN areas. In the IRS areas, the average percentage mortality for *An gambiae s.l* and *An. funestus s.l* was 34.5% and 95.6% respectively. In the ITN areas, the average percentage mortality was 83.7% and 89.0% respectively.

However, the numbers of the wild caught females of all malaria vectors tested were low. Sample sizes were below the recommended minimum of 300 which would allow for variability in the genetic structure of the successive F1 generations tested. Thus conclusions can be drawn about the presence of resistance but comparisons of resistance levels should be interpreted with caution.

The association of insecticide resistance in *Anopheles* species to agricultural use of insecticides has been reported (Mouchet 1988, Diabate et al. 2002), together with the resistance gene flow in malaria vectors (Lehmann et al. 1999, Pinto et al. 2002). In Zambia, the range of insecticides used for agricultural activities has increased recently, with a resultant potential increase in exposure of mosquito populations to a broader range of insecticides. Results from this study, demonstrate high level resistance to both DDT and deltamethrin in *An. gambiae* in some ITN areas. For example, in Myooye, an ITN area with intense cotton growing, *An. gambiae* and *An. funestus* showed resistance to both DDT and deltamethrin. *An. funestus* in Nyamankalo is resistant to both DDT and deltamethrin while, *An. gambiae* from the same locality is only resistant to deltamethrin. The detection of DDT resistance in ITN areas where no IRS programmes are currently being implemented can either be ascribed to the historical use of DDT or current excessive use of pyrethroids for agricultural purposes or the spatial distribution of resistance genes in vector populations. This suggests the presence of cross resistance conferred by target site,

kdr-type resistance and warrants robust further surveillance of *kdr* resistance in this region

It has been reported that low level resistance to insecticides as observed in areas with intense malaria vector control using IRS and ITNs suggests the presence of underlying biochemical and molecular mechanisms to be segregating in these populations (Collins et al. 2000, Hemingway et al. 1997). The historical use of DDT left behind the legacy of DDT-pyrethroid cross resistance in *An. gambiae* s.s in Africa (Martinez-Torres et al. 1998, Chandre et al. 1999) known as knockdown resistance (*kdr*). This mechanism is conferred by a single mutation in their common target site, the sodium channel, resulting in a leucine to phenylalanine (West Africa mutation) or a leucine to serine (East Africa mutation) change (Martinez-Torres et al. 1998, Ranson et al. 2000).

In Zambia, the intensive insecticide-based malaria vector control programme uses both DDT and pyrethroids for IRS. These products have a similar mode of action, as such the detection of resistance to both insecticides suggests the potential of *kdr*-based cross resistance between the two insecticides. Earlier data collected on the Copper belt province indicated the presence of a *kdr*-type mechanism together with altered AChE, GSTs and P450s segregating within the local *An. gambiae s.s* population (Mohloi 2006). In this study, west (leu-phe) *kdr* mechanism was detected in six localities. Of these areas, four: Kizhingezhinge in North western province, Chipulukusu, Mushili and Twapia in the Copperbelt province that have had extensive IRS programmes. Myooye and Chipepo in Central province are ITN areas with extensive cultivation of insecticide intensive crops particularly cotton which may have been responsible for *kdr* selection in west Africa (Martinez-Torres et al. 1998). The detection of the west kdr mutation at high frquencies in both IRS and ITN operational settings has got grave implications for the malaria control programme.

The presence of the west-type kdr in Zambia marks the most southernly documentation of this mutation and demonstrates the selection of resistance that has followed in the wake of scaled up vector control. This is likely to grossly compromise the efficacy of interventions and future malaria control efforts in the

country. It is not clear whether this resistance has arisen de novo in Zambia or whether it has spread from other locations in west Africa. Knock down resistance is controlled by recessive or semi-dominant genes. The detection of high west-type *kdr* allele frequency in the homozygous state in *An. gambiae s.s* in Zambia implies that this resistance is genetically recessive. Thus, increasing the opportunities of managing resistant populations. *An. funestus* was also found to be resistant to pyrethroids and DDT at high levels. To date sodium channel mutations in *An. funestus* have never been reported before. The DDT and pyrethroid cross resistance detected here could arise from two separate metabolic resistance mechanisms, GST and P450 (Hemingway et al. 2004) respectively, or may be the first instance of kdr type resistance in this species.

The detection of both pyrethroid and DDT resistance in *An. gambiae s.s* and the lack of any *kdr* mechanism in other areas suggest that an alternative resistance mechanism exists within the population. Metabolic mechanisms would involve glutathione-S-transferases (GSTs) or monooxygenases (P450s). The P450s primarily confer resistance to pyrethroids and carbamates (Brogdon and McAllister 1988) and their elevated levels of activity have been associated with pyrethroid resistance in *An. gambiae* (Vulule et al. 1994). The GSTs are involved in resistance to DDT, pyrethroids and organophosphates. GSTs are often elevated in DDT resistant insects (Prapanthadara et al., 1993; Grant and Hammock, 1992) and have also been studied in detail in *An. gambiae* (Enayati et al, 2005).

With the advent of more sophisticated biochemical and molecular assays for resistance detection it is now practicable to accurately analyze large numbers of insects for a range of insecticide resistance genes and monitor their changes over time (Penilla et al. 1998, Hemingway 1989, Hemingway et al. 1995). Biochemical mechanisms: altered acetylcholinesterase (AChE), glutathione *S*-transferase (GST), general esterase activity (pNPA; α- and β-naphthyl acetate), and monooxygenase (p450) could not be carried out in this study due to an inadequate cold chain to get samples to a suitable laboratory. New molecular-based techniques being developed will make detection of resistance mechanisms without the need for a cold chain easier in the future (Morgan et al. 2010).

Two further classes of insecticides carbamates and organophosphates are available for mainstream malaria control programs (Coosemans and Carnevale 1995, Walker 2000). The short half-lives of their current formulations, mean that they require two to three rounds of IRS per year. Combined, in some instances, with their expense, this can make these insecticides too costly for many malaria control programs, despite fewer reports of resistance (Coleman et al. 2006). No resistance was detected to organophosphates and carbamates in this study. However, the sample sizes (n<30) is small. More work is required to determine the resistance status to these insecticides if they are to be considered as alternatives for vector control.

The resistance reported here in *An. funestus* and *An. gambiae*, Zambia's major malaria vectors, is of major concern, as the current vector control policy is insecticide-based, and uses both DDT and pyrethroids for IRS and pyrethroid treated bed nets. Certain sections of the country are not amenable for IRS and are thus wholly dependent on ITNs use. Resistance has resulted in control failure in neighbouring countries, for example, Sharp and le Sueur (1996) reported the failure of malaria control in South Africa in 1996 due to pyrethroid-resistance selection in *An. funestus* and the reintroduction of *An. funestus* population from Mozambique into South Africa.

In Africa, there is mounting evidence of insecticide resistance that can potentially undermine IRS programmes (Coleman et al. 2008). The impact of *kdr* on IRS was significant in the malaria control programme on Bioko Island, Equatorial Guinea, as monitored through relative vector density resulting in a change from pyrethroid to carbamate for IRS (Sharp et al. 2007). This change was attributed to *kdr* but as the study did not monitor for metabolic resistance care needs to be taken interpreting this as due to *kdr* alone. Monitoring malaria cases in Kwa-Zulu Natal, South Africa, picked up the failure of pyrethroids in the IRS programme in the 1990s, resulting in DDT being reintroduced (Maharaj et al. 2005). In Mozambique the IRS policy changed from DDT to pyrethroid use in 1993, but due to high level pyrethroid resistance in *An. funestus* with lower levels detectable in *An. arabiensis*, there was a further change in policy to carbamates in 2000 (Coleman et al. 2008).

The spread of pyrethroid resistance may be critical for sustainability of ITNs, because this is currently the only insecticide group recommended for net impregnation. The operational impact of *kdr* on ITNs has been tested in experimental field trials albeit with conflicting results. While it has been shown that ITNs continue to provide individual protection despite *kdr* resistance in the vector population (Darriet et al. 2000, Henry et al. 2005, Dabire et al. 2006), an experimental hut trial in Côte d'Ivoire demonstrated a survival advantage for *kdr* resistant mosquitoes (Kolaczinski et al. 2000). More recent studies have demonstrated that *kdr* can undermine ITNs where the West African *kdr* mutation is high (Sharp et al. 2007, N'guessan et al. 2007) but the studies could not properly monitor metabolic resistance.

Although reductions in sporozoite rates were achieved despite the presence of *kdr* in Bioko Island (Sharp et al. 2007) , there are still uncertainties on the effect of *kdr* on the efficacy of vector control interventions (Protopopoff et al. 2008). As the impact of the East African *kdr* mutation on intervention also remains unknown (Protopopoff et al. 2008), there is a real need to scale these studies up into malaria control programmes.

High insecticide resistance selection pressure has been ascribed to both agricultural and public health activities. Mouchet (1988) and Lines (1988) reviewed the link between the emergences of resistance with the expansion of agricultural activities. Agricultural use of insecticides caused resistance in Central American *An. albimanus* (Brogdon et al. 1988). In Southern Mexico, the combined use of different classes of insecticides for agricultural spraying and DDT for anti-malaria house-spraying resulted in high levels of resistance to organochlorines, organophosphates, carbamates and pyrethroids in *An. albimanus* in the late 1970s (Penilla et al. 1998). Since then a reduction in agricultural insecticide used in this region has resulted in regression of the resistance to the point where it is barely detectable using standard WHO bioassays. In contrast DDT has been used for malaria control in this region for over 10 years and its continued use has maintained and increased the level of resistance to this insecticide. Equally, the impact of public health spraying on development of resistance has been exemplified in Haiti and in Sudan (Brogdon et al. 1988, Mouchet 1988). Furthermore, in Sri Lanka, resistance in one vector, *An.*

culicifacies, was characteristic of public health spraying, while resistance in another, *An. nigerrimus*, had a profile that indicated agricultural chemicals (Herath and Joshi 1986).

Population genetic studies of malaria vectors are an essential means of predicting and assessing the success of control measures (Pinto et al. 2002). Inferences on gene flow can also be useful predicators on the likelihood of the spread of insecticide-resistance genes (Collins et al. 2000). Several studies on the population structure of *An. gambiae s.s,* the most successful vector of malaria, have been conducted for better management of insecticide resistance (Collins and Besansky 1994, Lehmann et al. 2003, Besansky et al. 1997). Two genetic variants of *An. gambiae s.s* exist; the M and S forms (Favia et al. 1994, Wondji et al. 2002). However, the *kdr* resistance gene associated with pyrethroids and DDT resistance in this species was initially reported only in the molecular S form and was rare in the M form except for a few isolated localities (Elissa et al. 1993, Akogbeto and Yakoubou 1999, Chandre et al. 1999, Etang et al. 2006). Although the distribution of the members of the *An. gambiae* complex is well documented in Africa, the distribution of the molecular M and S forms and the *kdr* gene, however, is still being determined for much of Africa. Comparing the relative amounts of gene flow taking place in Zambia among populations should be the first step towards predicting the trajectory of introduced genes, particularly in areas with no history of DDT use.

The use of an insecticide until resistance becomes a limiting factor is rapidly eroding the number of available insecticides. In Zambia, a better management strategy may be the use of compounds in rotational or mosaic strategies (Mellon and Georghiou 1984, Curtis et al. 1993). Numerous mathematical models have been produced to determine the optimal strategies for resistance management (Greever and Georghiou 1979, Georghiou 1980, Tabashnik 1989). These models have been tested under laboratory but not field conditions due to practical difficulties of accurately assessing the changes in resistance gene frequencies associated with different patterns of insecticide use in large-scale field populations of insects (Taylor et al. 1983). However, large-scale field programme have been conducted in Southern Mexico to compare changes of resistance gene frequencies in the major malaria vector *An. albimanus* Wiedemann after repeated cycles of house-spraying for 3

years with 1) blanket long term use of a single insecticide, 2) spatial mosaic of two insecticide classes, or 3) annual rotation of three insecticide classes. Biological and biochemical assays showed that high level resistance development was reduced and kept at low levels by using rotations or mosaics schemes rather than the single insecticide regimes (Hemingway et al. 1997, Penilla et al. 1998).

The WHO criterion for resistance is that < 80% mortality post 24 h exposure indicates resistance (WHO, 1998). While WHO discriminating dosages, have shown the highest resistance frequencies for both DDT and deltamethrin in some sites with low or no resistance to these insecticides in other sites, it should be noted for bioassays that the WHO discriminating dosages are set at double the insecticide dose that a probit mortality/log dose regression predict would give 99.9% mortality of the least susceptible *Anopheles* mosquitoes from a range of mosquitoes tested. Hence, these bioassays are good indicators of the presence of significant levels of resistance (2-10-fold) in a mosquito population, but they cannot (with the potential exception of the dieldrin bioassays with 4% and 0.4% papers detecting RR and RS genotypes respectively) be used to monitor resistance gene frequencies accurately and often significantly underestimate the resistance present. Therefore, the bioassays alone do not provide an acceptable monitoring tool for low levels of resistance (Hemingway et al. 1997).

In this regard, the detection of confirmed and suspected levels of phenotypic resistance to all key insecticides for malaria control in both *An. funestus s.l* and *An. gambiae* s.*l* necessitates the urgent need for determining the underlying biochemical and molecular resistance mechanisms with the view of establishing a viable resistance management strategy for the malaria control programme in Zambia. An evidence-based and controlled rotation of insecticides is currently being planned to facilitate this, coupled to close monitoring of spatial and temporal resistance profiles of vectors using an established geographical information system (GIS)-based malaria decision support system (MDSS) as opposed to detecting its existence through operationally significant increases in disease transmission. The implementation of non-insecticide based strategies such as the use of bio-larvicides (Bti and IGRs) and environmental management is also being scaled up where applicable.

CHAPTER SIX

Discussion and Conclusion

Malaria remains a major cause of morbidity and mortality in sub-Saharan Africa (Snow et al. 2005), particularly in children under the age of five years and pregnant women (Gamble et al. 2006, Brooker et al. 2006). In response to this burden of disease, targets for malaria control, elimination and eradication have been established (WHO 2008, WHO 1993, Komatsu et al. 2007, WHO 2008). In order to reach these goals there is a need for continuous surveillance, monitoring and evaluation of malaria control programmes to make informed decisions and guide control efforts.

Malaria transmission is notably very heterogeneous even at the smallest scale (Van den Berg and Takken 2007). This is driven by several biological and environmental determinants suggesting the need for precise targeting. The relationship between malaria transmission intensity and disease burden, as well as monitoring of their changes, has been a topic of considerable debate (Molineaux 1997, Lengeler et al. 2007, Beier et al. 1999, Snow et al. 1997, Byass 2008). Moreover, different tools and strategies may be better suited to different transmission intensities for optimal control. Similarly, different methods (and combinations of methods) with differing provenance and characteristics are needed for measuring transmission at different levels (Hay et al. 2008).

Since malaria distribution is not homogeneous, much effort needs to be expended towards defining local spatial distribution of the disease (Hay *et al*., 1996) especially in areas preparing for malaria elimination. Following the increased funding for malaria control (Komatsu et al. 2007), particularly in sub-Saharan Africa (Nchinda 1998, Marsh 1998), insecticide based malaria vector control interventions are being scaled up in most endemic countries (WHO 2008). Information is essential to allow for adaptation of intervention policy, procedures and methods to optimize the impact of interventions and rationalize resources.

In Zambia, the initial deployment of vector control interventions, ITN (1999) and IRS (2000) was based on minimal empirical evidence. However, information gathered since then has allowed for more informed decisions to be made on targeting these interventions. The coverage of both LLINs and IRS has surpassed the internationally agreed upon targets of at least 80% by 2010, with the aim of

reducing malaria morbidity and mortality by 50% by 2010 (WHO 2009). Zambia, having achieved high coverage now needs to sustain these interventions and is moving towards malaria elimination (Chizema-Kawesha et al. 2010). Key to attain this goal is strengthening of surveillance and monitoring and evaluation, to better focus interventions on outstanding foci (Feachem et al. 2009).

Traditionally, the impact of malaria control interventions have been evaluated through several methods including repeated population-based surveys; parasite prevalence, malaria–specific mortality and all course mortality (WHO 2009). Recent empirical evidence, observed in the field, has demonstrated measurable impacts of specific interventions on the vector population, sporozoite rates or infectious reservoir including insecticide resistance (Macdonald 1957, Molineaux 1997, Killeen et al. 2000, Protopopoff et al. 2007, Sharp et al. 2007). While prevalence of parasites in children has been frequently used as a surrogate measure for malaria transmission intensity (Beier et al. 1999), the potential of routine surveillance data in evaluation studies have not been fully exploited (WHO 2009) including vector abundance, infectivity and insecticide resistance.

In Zambia, a malaria risk map generated from survey population based on asymptomatic parasitaemia (Chimumbwa 2003), compares well with climate-based predictive models (Nchinda 1998) and expert opinion (MoH 2000). This stratifies the country into four different malaria transmission zones (Chimumbwa 2003). Stratum 1 (0% to < 15%) in urban areas, stratum 2 (15% to 25%) in highland plateaux, stratum 3 (25% to 40%) in relatively arid areas and stratum 4 (> 45%) in hot riverine areas. This classification has been designated and used in Africa particularly in Kenya (Snow et al. 1997, Omumbo et al. 1998). This study was predominantly conducted in 17 sites of low (0% to < 15%) transmission and two sites of moderate transmission (15% to 25%) all of which are seasonal malaria transmission (Teklehaimanot et al. 1993, Taylor and Mutambu 1986) classified as meso- to hypo- endemic. Due to the low incidence of malaria all age groups are at risk although malaria related mortality is more concentrated in 5-7 years olds (Chimumbwa 2003).

Following effective vector control in Zambia, the malaria disease has fallen in the human population. Previous studies have been conducted in Zambia as population based surveys or hospital based routine surveillance with widely heterogeneous results (Sharp et al. 2002, McClean and Senthilselvan 2002, Utzinger et al. 2001, Chanda et al. 2009). In this study, the overall average *P. falciparum* prevalence in children between the ages of 1 and <15 years was below 10% implying extremely low transmission. Findings from nationally representative malaria indicator surveys conducted in Zambia (MoH 2006, MoH 2008) have shown superb reductions in the prevalence of parasite infection between 2006 and 2008. The number of in-patient malaria cases and deaths among children < 5 years of age decreased by 57% and 62% respectively (MoH 2008).

This low prevelance in Zambia has been achieved in part due to the scaled up coverage of vector control interventions (WHO 2009). Comparing between IRS and ITNs, data from both prevalence surveys and routine case surveillance (Cases, mortality rates and case fatality rates) indicate considerable overall reduction with more pronounced and better intervention effects for IRS than ITNs. This held true for comparing the two interventions between 2009 and 2010 with children from IRS houses receiving better protection (OR=0.04, P=0.06) than their counterparts in ITNs houses (OR=0.84, P=0.77). The number of cases due to malaria fell from 2007 to 2008 by 30.7% with a reduction in CFR of 61.7%. Again better intervention effects where observed for IRS (OR=0.37, P=0.005) than ITNs (OR=0.96, P=0.913). These findings are consistent with those of other studies conducted in low transmission settings (Nyarango et al. 2006, Guyatt et al. 2002, Roberts 1964, Curtis et al. 1999, Guyatt et al. 2002).

Despite the difference in efficacy, both IRS and ITNs have had a significant impact on prevalence in Zambia. However, where the interventions occur together an incremental protective effect occurs similar to that found in other studies (Rowland et al. 1997, Yadav et al. 1998, Lengeler 2004, Graves et al. 2008, Kleinschmidt et al. 2007). This combined effect of interventions has reduced malaria prevalence to low levels setting a scene for malaria elimination. The incremental impact of combining these interventions may help elimination of malaria in these low transmission areas.

Routine surveillance data has often proved inadequate for monitoring control programmes (Some et al. 1997), and has been supplanted by parasite prevalence surveys (Keating et al. 2009). However, this study suggests that combining parasite prevalence survey data with routine surveillance can help optimize impact assessment of interventions in low transmission intensity areas.

Conventional intervention deployment criteria prioritize children under the age of five and expecting mothers (MoH 2006). Age-specific comparison showed significant difference in intervention effect (P=0.015) on children below 5 years (OR=0.48) and older children 5 to 14 years (OR=0.75). This study validates the findings by Kleinschmidt et al (2006) that children from 5 to <15 years of age are more vulnerable than their under 5 counterparts in this setting. This challenges the widely held premise that children under the age of five are the most at risk (Baird et al. 1998, Kleinschmidt and Sharp 2001). Thus necessitating empirically driven age-specific deployment of interventions and suggesting that all children need to be protected in Zambia.

This difference in intervention effect could reflect the challenge of inconsistent bed net utilization and justifies the need for enhanced Information Education and Communication (IEC) and timely replenishment of worn out ITNs. Country-wide scale up of IRS in eligible areas, regardless of it being logistically more complex than ITNs, could also be considered depending on availability of resources. The overall reduction in mortality and morbidity in children as observed from both prevalence survey and routine surveillance data cannot exclusively be ascribed to vector control, as ACTs that are being implemented across the country (Sipilanyambe et al. 2008) contribute significantly to improved cure rates. The impact of ACTs has been enhanced with the improved treatment seeking behavior of people and lack of stock outs of ACTs in health facilities (Chanda et al. 2009). Equally, the intermittent presumptive treatment (IPT) in pregnancy has been scaled up country-wide (MoH 2006).

Importantly, the reliability of malaria prevalence surveys diminishes with declining prevalence, as the sample size become too big (Yekutiel 1960, WHO. 1971). Although routine surveillance systems have limitations (WHO 2009, Graves et al.

2008), the use of data from both malaria parasite prevalence survey and routine surveillance is important, particularly in areas where parasite rates have dropped below 5% (Molineaux et al. 1988, Pull 1972). The reduced malaria infection rates due to extensive control programmes have created zones that are potentially prone to malaria epidemics; all age-groups are vulnerable. To optimally assess the impact of interventions, substantial effort should be invested in improving the rigour and depth of passive and active surveillance data to compliment the population based parasite prevalence data (Hay et al. 2008, Molineaux et al. 1988, Pull 1972) and facilitate for empirically driven decision-making for future planning for malaria prevention and control.

The malaria control policy that strives towards a malaria free Zambia has facilitated for the homogenous coverage of malaria control interventions including vector control. This has created more areas with combined IRS and ITNs. The context of universal coverage of interventions invariably precludes the availability of localities devoid of interventions that could act as lucid control areas since people cannot be denied access to them. As such, it should be noted that non-intervention effect data are only obtainable from surveys.

The huge burden of malaria in sub-Saharan Africa is as a result of the presence of competent and efficient vectors; *An. gambiae* s.s, *An. arabiensis* and *An. funestus* (Gillies and Coetzee 1987, Gillies and De Meillon 1968) that co-exist in much of this region, including Zambia. These species differ in malaria transmission potential and bionomics (Gillies and Coetzee 1987, Bruce-Chwatt 1985, Coluzzi 1984, Fontenille and Simard 2004, DeMeillon 1937, Adams 1940, Watson 1953, Pielou 1947, Paterson 1963, Shelly 1973, Bransby-Williams 1979) requiring differences in control approach.

Due to their linear correlation with transmission (Molineaux 1997, Saul 1993, Killeen et al. 2000, Macdonald 1957), the direct impact of interventions on malaria transmission can be monitored by species density and infectivity (Sharp et al. 2007). The endophilic nature of *An. funestus* and *An. gambiae s.l* makes these species susceptible to both IRS and ITN to reduce abundance and sporozoite rates (Protopopoff et al. 2007, Sharp et al. 2007). In this study, both IRS and ITNs had the

biggest impact on abundance of *An. gambiae* s.s, and *An. funestus* compared to *An. arabiensis*. Although ITNs worked, IRS had more dramatic intervention effect on vector abundance than the ITNs, with no *An. gambiae s.s* trapped in IRS areas. This would account for the bigger impact on prevalence. Similar results of IRS having a more prompt and powerful impact than ITNs on species abundance has been observed before (Curtis et al. 1999, Guyatt et al. 2002). The apparent elimination of *An. gambiae* s.s in IRS areas and suppression of *An. funestus* and *An. arabiensis* to a minimal level, coupled to the absence of vector infectivity in both IRS and ITNs settings is striking. This effect of reducing abundance and infectivity of malaria vectors results in a community wide protection (Lengeler 2004, Killeen et al. 2006).

Previous studies conducted in Zambia from areas devoid of vector control interventions demonstrated presence of *P. falciparum* sporozoites in the three major vectors to varying degrees (Kent et al. 2007, Shelly 1973, Bransby-Williams 1979, Zahar 1985, Chimumbwa 2003, Siachinji and Mulenga 2002). However, following effective control no infectious mosquitoes have been identified. The monitoring of spatial and temporal impact of IRS and ITNs on the abundance and infectivity of major malaria vectors has facilitated for the calculation of the malaria transmission index in operational settings and thus identification of areas with limited or no transmission.

The lack of infectious mosquitoes observed in this study signifies a zero transmission in both IRS and ITNs operational areas following effective and consistent implementation. With 0 transmission levels, the basic reproduction rate remains at 0 which allows elimination of transmission and therefore the disease to take place. This study demonstrates that intensive implementation of IRS and ITNs in Zambia has resulted in marked decline in abundance and sporozoite rates of *An. gambiae s.s* and *An. funestus* in operational settings. Therefore, validating the premise that *An. gambiae s.s and An. funestus* are characteristically more amenable to control by these two interventions than *An. arabiensis* (Lengeler and Sharp 2003). This would also explain the low transmission levels (meso-to hypo-endemicity) of malaria in these areas. However, the persistent low parsitaemia in the human population indicate the presence of infectious mosquitoes and thus continued transmission. The absence of sporozoites could be ascribed to the low numbers of

vectors collected resulting from flaws in the exit trap method used. This indicates the need for their replacement with more robust collection tools. In addition, these findings validate the fact that vector control culminates in a shift in species composition, as reported previously (Shelly 1973, Bransby-Williams 1979, Lindsay et al. 1998).

Notably, identification of *An. nili* and *An. funestus-like* species in Zambia, as well as the presence of *An. rivulorum* is striking as it does not only increase our knowledge of their distribution range but also underscores the significance of species characterization. Further work to understand these species and their transmission potential is necessary.

While entomological monitoring and evaluation is essential for rational large scale malaria vector control exit window traps did have flaws in this low transmission setting. Non-compliance of householders became a big issue due to traps black colour that raised suspicions of Satanism. Alternative methods of monitoring of indoor vector abundance should be included to improve this monitoring system.

The predominance of *An. arabiensis* after effective vector control implies that the species may contribute to the perpetuation of malaria in the country, as demonstrated by the earlier studies (Shelly 1973, Bransby-Williams 1979, Zahar 1985). This may require scaled up activities to target this behaviourally facultative species directly. This may include larval source management using environmental management and larviciding (Singh et al. 1990, Smith et al. 1995), in the context of integrated vector management (Beier et al. 2008, Chanda et al. 2008).

The continued efficacy of refined contemporary malaria vector control tools that are primarily insecticide-based is threatened by the potential of insecticide resistance selection (Hemingway et al. 1997, Collins et al. 2000). The evidence of insecticide resistance operationally undermining malaria vector control programmes in Africa is mounting (Coleman et al. 2008, Sharp et al. 2007, N'guessan et al. 2007) and is invariably resulting in policy changes (Sharp et al. 2007, Maharaj et al. 2005, Coleman et al. 2008). Monitoring of resistance profiles of major vectors of the disease is essential.

This study detected high level resistance of both *An. gambiae s.l* and *An. funestus* to pyrethroids and DDT. There was great variation in the level of resistance between IRS and ITNs localities, with exceptionally higher level resistance detected in IRS areas compared to the ITNs ones. These results confirms other findings of resistance developing in major malaria vectors in the wake of extensive vector control (Sharp et al. 2007, Coleman et al. 2008, Protopopoff et al. 2007, Hemingway and Bates 2003). Conclusions can be drawn about the presence of resistance but comparisons of resistance levels should be interpreted with caution because of the low variability in the genetic structure of the tested samples of vector mosquitoes.

Pyrethroid-DDT cross resistance from a common knock down resistance (*kdr*) mechanism, has been reported in *An. gambiae* s.s in Africa (Ranson et al. 2000, Martinez-Torres et al. 1998, Chandre et al. 1999). In this study, Samples of *An. gambiae s.s* that were pyrethroid and DDT resistant were tested for east and west *kdr* mutations. The west (leu-phe) *kdr* mechanism was detected in four localities with extensive IRS programmes and in two ITN areas with extensive cultivation of insecticide intensive crops particularly cotton. This is the most southernly documented detection of this mutation and demonstrates the selection of resistance that has followed in the wake of scaled up vector control. However, it is not clear whether this resistance has arisen de novo in Zambia or whether it has spread from other locations in west Africa. The detection of the west *kdr* mutation at high frquencies in both IRS and ITN operational settings has got grave implications for the malaria control programme. This is likely to grossly compromise the efficacy of interventions and future malaria control efforts in the country. Knock down resistance is controlled by recessive or semi-dominant genes. The detection of high west-type *kdr* allele frequency in the homozygous state in Zambia also implies that this resistance is genetically recessive. Thus increasing the opportunities of managing the resistant populations.

The west-type *kdr* was detected in all areas with both DDT and pyrethroid resistance. The presence of kdr suggests that the cross resistance between DDT and pyrethroids is in part due to an altered sodium channel. Metabolic resistance mechanisms present in *An. gambiae s.s* (Awolola et al. 2003) would involve glutathione-S-transferases (GSTs) (Brogdon and McAllister 1988, Vulule et al.

1994) and/or monooxygenases (P450s) (Grant and Hammock 1992, Amenya et al. 2008). *An. funestus* was also found to be resistant to pyrethroids and DDT at high levels. To date there have been no reports of sodium channel mutations in *An. funestus.* The DDT and pyrethroid resistance detected here could arise from two separate metabolic resistance mechanisms, GST and P450 (Hemingway et al. 2004) respectively, or may possibly be the first instance of kdr type resistance in this species. More research is necessary to determine the actual underlying mechanisms. This is made easier with the advent of new molecular-based techniques (Morgan et al. 2010).

Resistance selection has been associated with agricultural use of insecticides (Penilla et al. 1998, Mouchet 1988, Diabate et al. 2002). This study did not indicate anything to the contrary, *An. gambiae s.l* and *An. funestus s.l* has shown high level resistance to both DDT and deltamethrin in ITN areas with intense cotton growing. The detection of DDT resistance in ITN areas with no ongoing IRS programmes is striking as it suggests the presence of cross resistance conferred by target site, kdr-type resistance and warrants robust further surveillance of kdr resistance in this region and conducting of gene flow studies (Lehmann et al. 1999, Pinto et al. 2002) to determine the geographical spread of this mutation.

In Mozambique the IRS policy changed from DDT to pyrethroid in 1993 but high level pyrethroid resistance in *An. funestus* with lower levels detectable in *An. arabiensis,* resulted in another policy change to carbamates in 2000 (Coleman et al. 2008). Monitoring malaria cases in Kwa-Zulu Natal, South Africa, picked up the failure of pyrethroids in the IRS programme in the 1990s due to P450 mediated pyrethroid-resistance selection in *An. funestus* (Sharp and le Sueur 1996) resulting in DDT being reintroduced (Maharaj et al. 2005). This was followed by a change in drug treatment policy (Barnes et al. 2005). In Bioko Island, Equatorial Guinea, the impact of *kdr* on IRS was significant in the malaria control programme as monitored through relative vector density resulting in a change from pyrethroid to carbamate for IRS (Sharp et al. 2007). The spread of pyrethroid resistance may be critical for sustainability of insecticide-treated bednets (ITNs), because this is currently the only insecticide group recommended for impregnation. Empirical studies have demonstrated that *kdr* can undermine ITNs where the West African kdr mutation is

high (Sharp et al. 2007, N'guessan et al. 2007).

The detection of resistance to DDT and pyrethroids in major vectors of malaria in Zambia has got grave implications for the malaria control programme. This is likely to compromise the efficacy of interventions and lead to the failure of IRS and possibly ITNs based control and result in increased malaria case load. During this study, prevalence of infection decreased between 2008 and 2009 but increased in 2010 in both IRS and ITN areas. This rebound in parasite prevalence could signify the beginning of control failure due to selection of insecticide resistance. This makes resistance monitoring essential for the malaria control programme.

Overall, there is need for further work to determine the underlying biochemical and molecular resistance mechanisms coupled with gene flow to assess the distribution of the resistant alleles and establishment of a viable resistance management strategy in Zambia. The evidence based and controlled rotation of insecticides currently being planned for in Zambia including the integration of non-insecticide based strategies such as the use of bio-larvicides (Bti and IGRs) and environmental management is necessary. Monitoring insecticide resistance mechanisms that occur within vector populations should be an essential component of a routine surveillance system of all insecticide-based malaria control programs.

The detected complete susceptibility to the only two other classes of insecticides, carbamates and organophosphates, available for mainstream malaria control programs (Coosemans and Carnevale 1995, Walker 2000) on the other hand could provide an opportunity for the control programme to switch to these classes for IRS. These findings will allow malaria control programme mangers to better utilize the limited resources on insecticides to which the malaria vectors are still susceptible. However, more work is required to determine resistance levels to these insecticides if they are to be considered as alternatives. Large scale trials have demonstrated that high level resistance development can be suppressed and maintained at low levels by using rotations or mosaics schemes rather than the single insecticide regimes (Hemingway et al. 1997, Penilla et al. 1998). Therefore, in Zambia a better management strategy may be the use of compounds in rotational or mosaic strategies (Mellon and Georghiou 1984, Curtis et al. 1993).

Following the effective implementation of vector control interventions, the resultant zero transmission should position the control programme to better achieve the goal of eliminating malaria in these low transmission areas. However, there is insecticide resistance building up following extensive use of insecticides for malaria control which could potentially impact on the ability to control the vector and lead to increases in abundance, transmission and thus undo the hard work and success attained thus far. Observed knowledge that mosquitoes are now being found resting inside sprayed houses in Zambia and the minimal increase in the prevalence of infection in children as observed in this study and the national malaria indicator survey for 2010 (MoH, 2010) point to this fact.

Even with high coverage of ITNs, parasitaemia in children was persistently high in Rufunsa in Lusaka province. This could be attributed to the high pyrethroid resistance levels detected in *An. funestus s.l* from this site. ITNs can also shift anopheline biting outdoors earlier in the evening (Magesa et al. 1991, Mbogo et al. 1996, Charlwood and Graves 1987). Although feeding and resting behavior was not the primary goal of this study, increased early evening outdoor biting was reported in Rufunsa and early evening outdoor biting *An. gambiae s.s* were collected in Chipulukusu an IRS area in the Copperbelt province with high pyrethroid and DDT resistance (Shinondo J. C. personal communication).

The results of this project indicate that there are many avenues that may be pursued for future studies. Research could focus on: detemination of inherent resistance mechanisms, population structure and malaria transmission potential of *An. nili* and *An. funestus*- like, and *An. rivulorum*, impact of insecticide resistance on malaria control interventions and vector bionomics.

Continuous surveillance, monitoring and evaluation of malaria interventions and their respective impacts on malaria burden is essential to increasing the efficiency and effectiveness of malaria control efforts and optimal utilization of limited resources (Goodman et al. 1999). An effective system for monitoring and evaluation and continuous surveillance requires integration of spatially and temporally explicit data for health information, intervention coverage and usage of entomological and epidemiological outcome indicators.

In this study, relative change in prevalence of infection, vector susceptibility to insecticides, and their abundance and transmission index over time has enabled measurement of spatial heterogeneity of trend or impact. The revealed trends and inter relationships have allowed the identification of areas with reduced parasitaemia and increased insecticide resistance thus demonstrating the impact of resistance on vector control. This will facilitate decision making and rational utilization of limited resources in a cost effective manner.

References

Abose, T., Y. Ye-Ebiyo, and al. E. Olana D. 1998. Re-orientation and Definition of the Role of Malaria Vector Control in Ethiopia: The Epidemiology and Control of Malaria with Special Emphasis on the Distribution, Behaviour and Susceptibility of Insecticides of Anopheline Vectors and Chloroquine Resistance in Zwai, Central Ethiopia and Other Areas. (WHO/MAL/981085) WHO, Geneva: 1-30.

Adams, P. C. G. 1940. Some observations on the flight of stained anophelines at Nkana, Northern Rhodesia. Ann.Trop.Med.Parasitol. 34-35.

Akogbeto, M. and S. Yakoubou. 1999. Resistance of malaria vectors to pyrethroids used for impregnated bednets in Benin. West Afr. Bull.Soc.Pathol.Exot. 92, 123-130.

Aldridge, W. N. 1993. The esterases: Perspectives and problems. Chemico Biological Interactions 87, 5-13.

Amenya, D. A., R. Naguran, T. C. Lo, H. Ranson, B. L. Spillings, O. R. Wood, B. D. Brooke, M. Coetzee, and L. L. Koekemoer. 2008. Over expression of a cytochrome P450 (CYP6P9) in a major African malaria vector, *Anopheles Funestus*, resistant to pyrethroids. *Insect Mol. Biol.* 17(1): 19-25.

Anderson, R. M. and R. M. May. 1991. *Infectious diseases of humans: dynamics and control*. Oxford and New York: Oxford University Press. ISBN 0198545991.

Andrewartha, H. G. and L. C. Birch. 1954. The distribution and abundance of animals. University of Chicago Press, Chicago.

Antonio-Nkondjio, C. Kerah Hinzoumbe, F. Simard, P. Awono-Ambene, T. Tchuinkam, and D. Fontenille. 2006. Complexity of the malaria vectorial system in Cameroon: Contribution of secondary vectors to malaria transmission. J Med Entomol. 43, 1215-1221.

Awolola, T. S., B. D. Brooke, L. L. Koekemoer, and M. Coetzee. 2003. Absence of the kdr mutation in the molecular 'M' form suggests different pyrethroid resistance mechanisms in the malaria vector mosquito *Anopheles gambiae* s.s. *Trop Med Int Health.* 8(5): 420-422.

Awolola, T. S., I. O. Oyewole, L. L. Koekemoer, and M. Coetzee. 2005. Identification of three members of the *Anopheles funestus* (Diptera: Culicidae) group and their role in malaria transmission in two ecological zones in Nigeria. *Trans. R. Soc. Trop. Med. Hyg.* 99(7): 525-531.

Awono-Ambene, P. Kengne, Simard F., C. Antonio-Nkondjio, and D. Fontenille. 2004. Description and bionomics of *Anopheles* (Cellia) *ovengensis* (Diptera: Culicidae), a new malaria vector species of the *Anopheles nili* group from south Cameroon. J Med Entomol. 41, 561-568.

Awono-Ambene, P., C. Antonio-Nkondjio, Toto J.C, Ndo C, Etang J, Fontenille D, and F. Simard. 2009. Epidemological importance of the *Anopheles nili* group of malaria vectors in equatorial villages of Cameroon, Central Africa. Sci Med Afr. 1, 13-20.

Ayad, H. and G. P. Georghiou. 1975. Resistance to organophosphates and carbamates in *Anopheles albimanus* based on reduced sensitivity to acetylcholinesterase. J.Econ.Entomol. 68, 295-297.

Ayala, D., Carlo Costantini, Ose K, G. C. Kamdem, C. Antonio-Nkondjio, J. P. Agbor, P. Awono-Ambene, D. Fontenille, and F. Simard. 2009. Habitat suitability and ecological niche profile of major malaria vectors in Cameroon. Malar J. 8, 307.

Ayala, F. J. and M. Coluzzi. 2005. Chromosome speciation: humans, Drosophila, and mosquitoes. *Proc. Natl. Acad. Sci. U. S. A.* 102 Suppl 1:6535-42. Epub@2005 Apr 25.: 6535-6542.

Baird, J. K., S. Masbar, H. Basri, S. Tirtokusumo, B. Subianto, and S. L. Hoffman. 1998. Age-dependent susceptibility to severe disease with primary exposure to *Plasmodium falciparum*. *J Infect. Dis.* 178(2): 592-595.

Bangs, M. J., S. Rusmiarto, Y. R. Gionar, A. S. Chan, K. Dave, and J. R. Ryan. 2002. Evaluation of a dipstick malaria sporozoite panel assay for detection of naturally infected mosquitoes. *J Med Entomol.* 39(2): 324-330.

Barat, L. M., B. Himonga, S. Nkunika, M. Ettling, T. K. Ruebush, W. Kapelwa, and P. B. Bloland. 1998. A systematic approach to the development of a rational malaria treatment policy in Zambia. *Trop Med Int Health.* 3(7): 535-542.

Barnes, K. I., P. Chanda, and B. G. Ab. 2009. Impact of the large-scale deployment of artemether/lumefantrine on the malaria disease burden in Africa: case studies of South Africa, Zambia and Ethiopia. *Malar J.* 8 Suppl 1:S8.: S8.

Barnes, K. I., D. N. Durrheim, F. Little, A. Jackson, U. Mehta, E. Allen, S. S. Dlamini, J. Tsoka, B. Bredenkamp, D. J. Mthembu, N. J. White, and B. L. Sharp. 2005. Effect of artemether-lumefantrine policy and improved vector control on malaria burden in KwaZulu-Natal, South Africa. *PLoS. Med.* 2(11): e330.

Barnes, K. I., F. Little, A. Mabuza, N. Mngomezulu, J. Govere, D. Durrheim, C. Roper, B. Watkins, and N. J. White. 2008. Increased gametocytemia after treatment: an early parasitological indicator of emerging sulfadoxine-pyrimethamine resistance in falciparum malaria. *J. Infect. Dis.* 197(11): 1605-1613.

Bass C., D. Nikou, A. M. Blagborough, J.Vontas, R.E. Sinden, M.S. Williams, L.M. Field. 2008. PCR-based detection of *Plasmodium* in *Anopheles* mosquitoes: a comparison of a new high-throughput assay with existing methods. Malar J 7:177.

Baton, L. A. and L. C. Ranford-Cartwright. 2005. Spreading the seeds of million-murdering death: metamorphoses of malaria in the mosquito. *Trends Parasitol.* 21(12): 573-580.

Bayoh, M. N. and S. W. Lindsay. 2003. Effect of temperature on the development of the aquatic stages of *Anopheles gambiae* sensu stricto (Diptera: Culicidae). *Bull. Entomol. Res.* 93(5): 375-381.

Bayoh, M. N. and S. W. Lindsay. 2004. Temperature-related duration of aquatic stages of the Afrotropical malaria vector mosquito *Anopheles gambiae* in the laboratory. *Med. Vet. Entomol.* 18(2): 174-179.

Beier, J. C., G. F. Killen, and J. I. Githure. 1999. Short report: entomologic innoculation rates and *Plasmodium falciparum* malaria preva;ence in Africa. Am J Trop Med Hyg 61, 109-113.

Beier, J. C., J. Keating, J. I. Githure, M. B. Macdonald, D. E. Impoinvil, and R. J. Novak. 2008. Integrated vector management for malaria control. *Malar. J.* 7 Suppl 1:S4.: S4.

Beier, J. C. and J. K. Koros. 1991. Visual assessment of sporozoite and bloodmeal ELISA samples in malaria field studies. Journal of Medical Entomology. 28, 805-808.

Beier, J. C., C. N. Oster, F. K. Onyango, J. D. Bales, J. A. Sherwood, P. V. Perkins, D. K. Chumo, D. V. Koech, R. E. Whitmire, C. R. Roberts, and . 1994. *Plasmodium falciparum* incidence relative to entomologic inoculation rates at a site proposed for testing malaria vaccines in western Kenya. *Am J Trop Med Hyg.* 50(5): 529-536.

Beier, J. C., P. V. Perkins, J. K. Koros, F. K. F.K.Onyango, T. P. Gargan, R. A. Wirtz, D. K. D.K.Koech, and Roberts.C.R. 1990. Malaria sporozoite detection by dissection and ELISA to assess infectivity of Afrotropical *Anopheles* (Diptera: Culicidae). J.Med.Entomol. 27, 377-384.

Bell, D. and R. W. Peeling. 2006. Evaluation of rapid diagnostic tests: malaria. *Nat. Rev. Microbiol.* 4(9 Suppl): S34-S38.

Bermudez, I., C. A. Hawkins, A. M. Taylor, and D. J. Beadle. 1991. Actions of insecticides on the insect GABA receptor complex. J.Recept.Res. 11, 221-232.

Berzosa, P. J., J. Cano, J. Roche, J. M. Rubio, L. Garcia, E. Moyano, A. Guerra, J. C. Mateos, V. Petrarca, V. D. Rosario, and A. Benito. 2002. Malaria vectors in Bioko Island (Equatorial Guinea): PCR determination of the members of Anopheles gambiae Giles complex (Diptera: Culicidae) and pyrethroid knockdown resistance (kdr) in An. gambiae sensu stricto. *J. Vector. Ecol.* 27(1): 102-106.

Besansky, N. J., C. A. Hill, and C. Costantini. 2004. No accounting for taste: host preference in malaria vectors. *Trends Parasitol.* 20(6): 249-251.

Besansky, N. J., T. Lehmann, G. T. Fahey, D. Fontenille, L. E. Braack, W. A. Hawley, and F. H. Collins. 1997. Patterns of mitochondrial variation within and between African malaria vectors, Anopheles gambiae and An. arabiensis, suggest extensive gene flow. *Genetics.* 147(4): 1817-1828.

Bhattarai, A., A. S. Ali, S. P. Kachur, A. Martensson, A. K. Abbas, R. Khatib, A. W. Al-Mafazy, M. Ramsan, G. Rotllant, J. F. Gerstenmaier, F. Molteni, S. Abdulla, S.

M. Montgomery, A. Kaneko, and A. Bjorkman. 2007. Impact of artemisinin-based combination therapy and insecticide-treated nets on malaria burden in Zanzibar. *PLoS. Med.* 4(11): e309.

Bigoga, J. D., L. Manga, V. P. Titanji, M. Coetzee, and R. G. Leke. 2007. Malaria vectors and transmission dynamics in coastal south-western Cameroon. *Malar. J.* 6:5.: 5.

Bijl, H. M., J. Kager, D. W. Koetsier, and T. S. van der Werf. 2000. Chloroquine- and sulfadoxine-pyrimethamine-resistant Falciparum malaria in vivo - a pilot study in rural Zambia. *Trop Med Int Health.* 5(10): 692-695.

Bloomquist, J. R. 1994. Cyclodiene resistance at the insect GABA receptor chloride channel complex confers broad cross-resistance to convulsants and experimental phenylpyrazole insecticides. Arch.Insect Biochem.Physiol. 26, 69-79.

Boete, C. and J. C. Koella. 2003. Evolutionary ideas about genetically manipulated mosquitoes and malaria control. *Trends Parasitol.* 19(1): 32-38.

Bogh, C., S. E. Clarke, M. Jawara, C. J. Thomas, and S. W. Lindsay. 2003. Localized breeding of the *Anopheles gambiae* complex (Diptera: Culicidae) along the River Gambia, West Africa. Bull Entomol Res. 93, 279-287.

Bogh, C., S. W. Lindsay, S. E. Clarke, A. Dean, and M. e. al. Jawara. 2007. High spatial resolution mapping of malaria transmission risk in the Gambia, west Africa, using LANDSAT TM satellite imagery. Am J Trop Med Hyg. 76, 875-881.

Bogh, C., E. M. Pedersen, D. A. Mukoko, and J. H. Ouma. 1998. Permethrin-impregnated bed net effects on resting and feeding behaviour of lymphatic filariasis vector mosquitoes in Kenya. Med Vet Entomol. 12, 52-59.

Booman, M., B. L. Sharp, C. L. Martin, B. Manjate, J. J. La Grange, and D. N. Durrheim. 2003. Enhancing malaria control using a computerised management system in southern Africa. *Malar. J.* 2:13. Epub@2003 Jun 6.: 13.

Boudin, C., Robert V, P. Carnevale, and P. Ambroise-Thomas. 1992. Epidemiology of *Plasmodium falciparum* in a rice field and a savanna area in Burkina Faso. Comparative study on the acquired immunoprotection in native populations. Acta Trop. 51, 103-111.

Bourguet, D., A. Genissel, and M. Raymond. 2000. Insecticide resistance and dominance levels. *J Econ. Entomol.* 93(6): 1588-1595.

Bourguet, D., M. Prout, and M. Raymond. 1996. Dominance of insecticide resistance presents a plastic response. *Genetics.* 143(1): 407-416.

Bransby-Williams, W. 1979. House catches of adult *Anopheles gambiae* species B in two areas of Zambia. East African Medical Journal. 56, 557-561.

Breman, J. G., M. S. Alilio, and A. Mills. 2004. Conquering the intolerable burden of malaria: what's new, what's needed: a summary. *Am J Trop Med Hyg.* 71(2 Suppl): 1-15.

Breman, J. G., A. Egan, and G. T. Keusch. 2001. The intolerable burden of malaria: a new look at the numbers. *Am J Trop Med Hyg.* 64(1-2 Suppl): iv-vii.

Breman, J. G. and W. P. O'Meara. 2005. Intermittent preventive treatment for malaria in infants: moving forward, cautiously. *J Infect. Dis.* 192(11): 1869-1871.

Brengues, C., N. J. Hawkes, F. Chandre, L. McCarroll, S. Duchon, P. Guillet, S. Manguin, J. C. Morgan, and J. Hemingway. 2003. Pyrethroid and DDT cross-resistance in *Aedes aegypti* is correlated with novel mutations in the voltage-gated sodium channel gene. Med.Vet.Entomol. 17, 87-94.

Brogdon, W. G. and A. M. Barber. 1990. Fenitrothion-deltamethrin cross-resistance confered by esterases in Guatemalan *Anopheles albimanus*. Pestic.Biochem.Physiol. 37, 130-139.

Brogdon, W. G. and J. C. McAllister. 1988. Insecticide resistance and vector control. Emerging Infectious Diseases 4, 605-613.

Brogdon, W. G., J. C. McAllister, and J. Vulule. 1997. Heme peroxidase activity measured in single mosquitoes identifies individuals expressing the elevated oxidase mechanism for insecticide resistance. J.Am.Mosq.Control.Assoc. 13, 233-237.

Brogdon, W. G., J. H. Hobbs, J. Y. St, J. R. Jacques, and L. B. Charles. 1988. Microplate assay analysis of reduced fenitrothion susceptibility in Haitian Anopheles albimanus. *J Am Mosq Control Assoc.* 4(2): 152-158.

Brooker, S., T. Leslie, K. Kolaczinski, E. Mohsen, N. Mehboob, S. Saleheen, J. Khudonazarov, T. Freeman, A. Clements, M. Rowland, and J. Kolaczinski. 2006. Spatial epidemiology of Plasmodium vivax, Afghanistan. *Emerg. Infect Dis.* 12(10): 1600-1602.

Brown, A. W. A. 1978. Ecology of pesticides. John Wiley and Sons, New York.

Brown, A. W. A. 1986. Insecticide resistance in mosquitoes: a pragmatic review. J.Am.Mosq.Control Assoc. 2, 123-140.

Bruce-Chwatt. 1985.Essential Malariology. 2nd edition. John Willey and Sons. New York. 166-179.

Brunhes, J., LeGoff G, and Geoffroy B. 1999. Afro-tropical anopheline mosquitoes:description of three new species: *Anopheles carnevalei* sp. nov., *An. hervyi* sp. nov and *An. dualaensis* sp. nov., and resurrection of *An. rageaui* Mattingly and Adam. J Am Mosq Control Assoc. 15, 552-558.

Bryan, J. H., M. A. Di Deco, V. Petraca, and M. Coluzzi. 1982.Inversion polymorphism and incipient speciation in *Anopheles gambiae* s.s in Gambia, West Africa. Genetica 59, 167-176.

Burkot, T. R., J. L. Williams, and I. Schneider. 1984. Identification of *Plasmodium falciparum*-infected mosquitoes by a double antibody enzyme-linked immunosorbent assay. American Journal of Tropical Medicine and Hygiene. 33, 883-788.

Byass, P. 2008. Making sense of long-term changes in malaria. *Lancet.* 372(9649): 1523-1525.

Campbell, P. M., J. F. Trott, C. Claudianos, K. A. Smyth, R. J. Russell, and J. G. Oakeshott. 1997.Biochemistry of esterases associated with organophosphorus resistance in Lucilia cuprina with comparisons to putative orthologues in other Diptera. Biochem Genet. 35, 17-40.

Cano, J., M. A. Descalzo, M. Moreno, Z. Chen, and S. e. al. Nzambo. 2006. Spatial variability in the density, distribution and vectorial capacity of anopheline species in a high transmission village (Equatorial Guinea). Malar J. 5, 21.

Carino, F. A., J. F. Koener, F. W. J. Plapp, and R. Feyereisen. 1994. Constitutive overexpression of the cytochrome P450 gene Cyp6A1 in a house fly strain with metabolic resistance to insecticides. Insect Biochem Mol Biol. 24, 411-418.

Carnevale, P., L. G. G. , J. C. oto, and obert V. 1992. *Anopheles nili* as the main vector of human malaria in villages of southern Cameroon. Med Vet Entomol. 6, 135-138.

Carnevale, P., V. Robert, C. Boudin, J. M. Halna, L. Pazart, P. Gazin, A. Richard, and J. Mouchet. 1988. [Control of malaria using mosquito nets impregnated with pyrethroids in Burkina Faso]. Bull Soc Pathol Exot. Filiales. 81(5): 832-846.

Carter, T. 2004. DDT: Malaria Answer in Africa? 2 million die each year from disease.The Washington Times.

Casida, J. E. and G. B. Quistad. 1998. Golden age of insecticide research: past, present, or future? *Annu. Rev. Entomol.* 43:1-16.: 1-16.

Casimiro, S., M. Coleman, P. Mohloai, J. Hemingway, and B. Sharp. 2006. Insecticide resistance in *Anopheles funestus* (Diptera: Culicidae) from Mozambique. *J. Med. Entomol.* 43(2): 267-275.

Casimiro, S. L., J. Hemingway, B. L. Sharp, and M. Coleman. 2007. Monitoring the operational impact of insecticide usage for malaria control on *Anopheles funestus* from Mozambique. *Malar. J.* 6:142.: 142.

Cattani, J. A., J. S. Moir, F. D. Gibson, M. Ginny, J. Paino, W. Davidson, and M. P. Alpers. 2005. Small-area variations in the epidemiology of malaria in Madang Province. 1986. *P. N. G. Med J.* 48(1-2): 95-101.

Ceesay, S. J., C. Casals-Pascual, J. Erskine, S. E. Anya, N. O. Duah, A. J. Fulford, S. S. Sesay, I. Abubakar, S. Dunyo, O. Sey, A. Palmer, M. Fofana, T. Corrah, K. A. Bojang, H. C. Whittle, B. M. Greenwood, and D. J. Conway. 2008. Changes in malaria indices between 1999 and 2007 in The Gambia: a retrospective analysis. *Lancet.* 372(9649): 1545-1554.

Chambers, G. M. and M. J. Klowden. 1990. Correlation of nutritional reserves with a critical weight for pupaion in larval *Aedes aegypti* mosquitoes. J Am Mosq Control Assoc. 3, 394-399.

Chanda, E. 2007. The transmission attributes of peri-urban malaria in Lusaka, Zambia. A Masters of Science (MSc) Thesis, University of Zambia.Lusaka, Zambia.

Chanda, E., F. Masaninga, M. Coleman, C. Sikaala, C. Katebe, M. MacDonald, K. S. Baboo, J. Govere, and L. Manga**.** 2008**.** Integrated vector management: the Zambian experience. *Malar. J.* 7:164.: 164.

Chanda, P., B. Hamainza, S. Mulenga, V. Chalwe, C. Msiska, and E. Chizema-Kawesha**.** 2009**.** Early results of integrated malaria control and implications for the management of fever in under-five children at a peripheral health facility: a case study of Chongwe rural health centre in Zambia. *Malar. J.* 8:49.: 49.

Chandre, F., F. Darriet, Manguin S, C. Brengues, P. Carnavale, and P. Guillet**.** 1999. Pyrethroid cross resistance spectrum among populations of *Anopheles gambiae s.s.* from Cote d'Ivoire. Journal of the American Mosquito Control Association. 15, 53-59.

Charlwood, J. D., W. D. Alecrim, N. Fe, J. Mangabeira, and V. J. Martins**.** 1995**.** A field trial with Lambda-cyhalothrin (ICON) for the intradomiciliary control of malaria transmitted by *Anopheles darlingi* root in Rondonia, Brazil. *Acta Trop.* 60(1): 3-13.

Charlwood, J. D. and P. M. Graves**.** 1987**.** The effect of permethrin-impregnated bednets on a population of *Anopheles farauti* in coastal Papua New Guinea. *Med Vet Entomol.* 1(3): 319-327.

Charlwood, J. D., M. Qassim, E. I. Elnsur, M. Donnelly, V. Petrarca, P. F. Billingsley, J. Pinto, and T. Smith**.** 2001**.** The impact of indoor residual spraying with malathion on malaria in refugee camps in eastern Sudan. *Acta Trop.* 80(1): 1-8.

Charlwood, J. D., T. Smith, E. Lyimo, A. Y. Kitua, H. Masanja, M. Booth, P. L. Alonso, and M. Tanner**.** 1998**.** Incidence of *Plasmodium falciparum* infection in infants in relation to exposure to sporozoite-infected anophelines. *Am J Trop Med Hyg.* 59(2): 243-251.

Chimumbwa, J. M. 2003.The epidemiology of malaria in Zambia. A Doctor of Philosophy (PhD) Thesis, University of Kwazulu Natal, South Africa.

Chinery, W. A. 1984**.** Effects of ecological changes on the malaria vectors Anopheles funestus and the Anopheles gambiae complex of mosquitoes in Accra, Ghana. *J. Trop. Med. Hyg.* 87(2): 75-81.

Chizema-Kawesha, E., J. M. Miller, R. W. Steketee, V. M. Mukonka, C. Mukuka, A. D. Mohamed, S. K. Miti, and C. C. Campbell**.** 2010**.** Scaling up malaria control in Zambia: progress and impact 2005-2008. *Am J Trop Med Hyg.* 83(3): 480-488.

Clark, A. G. and N. A. Shamaan**.** 1984.Evidence that DDTdehydrochlorinase from the house fly is a glutathione Stransferase. Pest Biochem Physiol. 22, 249-261.

Clements, A. N. 1992. The biology of mosquitoes. In. Development, nutrition and reproduction. London Chapman and Hall, London. 1.

Coetzee, M. 2004. Distribution of the African malaria vectors of the Anopheles gambiae complex. *Am J Trop Med Hyg.* 70(2): 103-104.

Coetzee, M., M. Craig, and S. D. le. 2000. Distribution of African malaria mosquitoes belonging to the Anopheles gambiae complex. *Parasitol. Today.* 16(2): 74-77.

Cohuet, A., F. Simard, C. S. Wondji, C. Antonio-Nkondjio, P. Awono-Ambene, and et al. 2004. High malaria transmission intensity due to *Anopheles funestus* (Diptera: Culicidae) in a village of savannah-forest transition area in Cameroon. J Med Entomol. 5, 901-905.

Cohuet, A., F. Simard, J. C. Toto, P. Kengne, M. Coetzee, and D. Fontenille. 2003. Species identification within the Anopheles funestus group of malaria vectors in Cameroon and evidence for a new species. *Am J Trop Med Hyg.* 69(2): 200-205.

Coleman, M. and J. Hemingway. 2007. Insecticide resistance monitoring and evaluation in disease transmitting mosquitoes. J.Pestic.Sci 32, 69-76.

Coleman, M., S. Casimiro, J. Hemingway, and B. Sharp. 2008. Operational impact of DDT reintroduction for malaria control on Anopheles arabiensis in Mozambique. *J. Med. Entomol.* 45(5): 885-890.

Coleman, M., B. Sharp, I. Seocharan, and J. Hemingway. 2006. Developing an evidence-based decision support system for rational insecticide choice in the control of African malaria vectors. *J. Med. Entomol.* 43(4): 663-668.

Collins, F. H., L. Kamau, H. A. Ranson, and J. M. Vulule. 2000. Molecular entomology and prospects for malaria control. *Bull. World Health Organ.* 78(12): 1412-1423.

Collins, F. H. and S. M. Paskewitz. 1995. Malaria: current and future prospects for control. *Annu. Rev. Entomol.* 40:195-219.: 195-219.

Collins, H. H. and N. J. Besansky. 1994. Vector biology and control of malaria in Africa. Science 264, 1874-1875.

Coluzzi, M. 1984. Heterogeneities of the malaria vectorial system in tropical Africa and their significance in malaria epidemiology and control. *Bull. World Health Organ.* 62 Suppl:107-13.: 107-113.

Coluzzi, M., V. Petrarca, and M. A. Di Deco. 1985. Chromozomal inversion intergradation and incipient speciation in Anopheles gambiae. Bollettino di Zoologia 52, 45-63.

Coluzzi, M., A. Sabatini, T. A. Della, M. A. Di Deco, and V. Petrarca. 2002. A polytene chromosome analysis of the Anopheles gambiae species complex. *Science.* 298(5597): 1415-1418.

Coluzzi, M., A. Sabatini, V. Petrarca, and M. A. Di Deco. 1979. Chromosomal differentiation and adaptation to human environments in the Anopheles gambiae complex. *Trans. R. Soc. Trop. Med. Hyg.* 73(5): 483-497.

Comins, H. 1986. Tactics for resistance management using multiple pesticides. Agric.Ecosyt.Environ. 16, 129-148.

Connor, S. J., S. Flasse, A. erryman, and M. C. homson. 1997. The contribution of satellite derived information to malaria stratification monitoring and early warning.World Health Organization: Geneva.

Coosemans, M. and P. Carnevale. 1995. Malaria vector control: a critical review on chemical methods and insecticides. *Ann. Soc. Belg. Med. Trop.* 75(1): 13-31.

Craig, M. H., R. W. Snow, and D. le Sueur. 1999. A climate-based distribution model of malaria transmssion in sub-Saharan Africa. Parasitol Today. 15, 105-111.

Craig, M. H., B. L. Bredenkamp, C. H. Williams, E. J. Rossouw, V. J. Kelly, I. Kleinschmidt, A. Martineau, and G. F. Henry. 2002. Field and laboratory comparative evaluation of ten rapid malaria diagnostic tests. *Trans. R. Soc Trop Med Hyg.* 96(3): 258-265.

CSO. 2000. Central Statistical Office, Zambia National Census Report 2000.

CSO. 2007. Zambia Central Statistics Office,Ministry of Health, and ORC Macro.2003. Zambia Demographic and Health Survey 2007.

Cuamba, N., K. S. Choi, and H. Townson. 2006. Malaria vectors in Angola: distribution of species and molecular forms of the Anopheles gambiae complex, their pyrethroid insecticide knockdown resistance (kdr) status and Plasmodium falciparum sporozoite rates. *Malar. J.* 5:2.: 2.

Curtis, C. F. and G. Davidson. 1981.Population genetics aspects of insecticide resistance in mosquitoes. In: Cytogenetics and Genetics of Vectors. Eds. R. Pal, J.B. Kitzmiller and T. Kanda.

Curtis, C. F. 1978. Genetic sex separation in Anopheles arabiensis and the production of sterile hybrids. *Bull. World Health Organ.* 56(3): 453-454.

Curtis, C. F., N. Hill, and S. H. Kasim. 1993. Are there effective resistance management strategies for vectors of human disease? Biological Journal of the Linnean Society. 48, 3-18.

Curtis, C. F. and J. D. Lines. 1985. Impregnated fabrics against malaria mosquitoes. *Parasitol Today.* 1(5): 147.

Curtis, C. F., J. E. Miller, M. H. Hodjati, J. H. Kolaczinski, and I. Kasumba. 1998. Can anything be done to maintain the effectiveness of pyrethroid-impregnated bednets against malaria vectors? *Philos. Trans. R. Soc. Lond B Biol. Sci.* 353(1376): 1769-1775.

Curtis, C. F. and A. E. Mnzava. 2000. Comparison of house spraying and insecticide-treated nets for malaria control. *Bull World Health Organ.* 78(12): 1389-1400.

Curtis, C. F., H. V. Pates, W. Takken, C. A. Maxwell, J. Myamba, A. Priestman, O.

Akinpelu, A. M. Yayo, and J. T. Hu**.** 1999**.** Biological problems with the replacement of a vector population by Plasmodium-refractory mosquitoes. *Parassitologia.* 41(1-3): 479-481.

Curtis, C. F. and H. Townson**.** 1998**.** Malaria: existing methods of vector control and molecular entomology. *Br. Med Bull.* 54(2): 311-325.

Cuzin-Ouattara, N., A. H. A. Van den Broek, and A. Habluetzel**.** 1999. Wide-scale installation of insecticide-treated curtains confers high levels of protection against malaria transmission in a hyperendemic area of Burkina Faso. Trans R Soc Trop

Cygler, M., J. D. Schrag, J. L. Susman, M. Harel, I. Silman, M. K. Gentry, and B. P. Doctor**.** 1993. Relationship between sequence conservation and 3-dimensional structure in a large family of esterases, lipases, and related proteins. Prot.Science 2, 366-382.

D'Alessandro, U., B. O. Olaleye, W. McGuire, M. C. Thomson, P. Langerock, S. Bennett, and B. M. Greenwood**.** 1995**.** A comparison of the efficacy of insecticide-treated and untreated bed nets in preventing malaria in Gambian children. *Trans. R Soc Trop Med Hyg.* 89(6): 596-598.

Daash, A., A. Srivastava, B. N. Nagpal, R. Saxena, and S. K. Gupta**.** 2009**.** Geographical information system (GIS) in decision support to control malaria--a case study of Koraput district in Orissa, India. *J Vector Borne. Dis.* 46(1): 72-74.

Dabire, R. K., A. Diabate, T. Baldet, L. Pare-Toe, R. T. Guiguemde, J. B. Ouedraogo, and O. Skovmand**.** 2006**.** Personal protection of long lasting insecticide-treated nets in areas of Anopheles gambiae s.s. resistance to pyrethroids. *Malar J.* 5:12.: 12.

Daly, H., J. T. Doyen, and A. H. I. Purcell**.** 1998. Introduction to insect biology and diversity, 2nd edition. Oxford University Press. New York, New York. Chapter 14, Pages 279-300.

Darriet, F., R. N'guessan, A. A. Koffi, L. Konan, J. M. Doannio, F. Chandre, and P. Carnevale**.** 2000**.** [Impact of pyrethrin resistance on the efficacity of impregnated mosquito nets in the prevention of malaria: results of tests in experimental cases with deltamethrin SC]. *Bull Soc Pathol Exot.* 93(2): 131-134.

Darsie, R. and R. Ward**.** 2005. Systematics. In Darsie R, Ward R, eds, *Identification and Geographical Distribution of the Mosquitoes of North America, north of Mexico*, 2nd ed. 1-6. University Press of Florida, Gainesville.

Dary, O., G. P. Georghiou, E. Parsons, and N. Pasteur**.** 1990**.** Microplate adaptation of Gomori's assay for quantitative determination of general esterase activity in single insects. *J Econ. Entomol.* 83(6): 2187-2192.

David, J. P., C. Strode, J. Vontas, D. Nikou, A. Vaughan, P. M. Pignatelli, C. Louis, J. Hemingway, and H. Ranson**.** 2005**.** The *Anopheles gambiae* detoxification chip: a highly specific microarray to study metabolic-based insecticide resistance in malaria vectors. *Proc. Natl. Acad. Sci. U. S. A.* 102(11): 4080-4084.

Davidson, G. and G. B. White**.** 1972**.** The crossing characteristics of a new, sixth species in the *Anopheles gambiae* complex. *Trans. R. Soc Trop Med Hyg.* 66(4): 531-532.

De Meillon, B., G. J. Van Eeden, L. Coetzee, M. Coetzee, Meiswinkel R, Du ToitLN, and Hansford CF. 1977. Observations on a species of the *Anopheles funestus* subgroup, a suspected exophilic vector of malaria parasites in northeastern Transvaal, South Africa. Mosq News. 37, 657-661.

de Savigny, D. and F. Binka**.** 2004**.** Monitoring future impact on malaria burden in sub-saharan Africa. *Am J Trop Med Hyg.* 71(2 Suppl): 224-231.

Della Torre, A., C. Costantini, N. J. Besansky, A. Caccone, V. Petrarca, J. R. Powell, and M. and Coluzzi**.** 2002. Speciation within *Anopheles gambiae* - the glass is half full. Science. 298, 115-117.

Della Torre, A., C. Fanello, M. Akogbeto, and et al**.** 2001.Molecular evidence of incipient speciation within *Anopheles gambiae s.s* in West Africa. Insecti Molecular Biology 10, 9-18.

DeMeillon, B. 1937. A note on *An. gambiae* and *An. funestus* in Northern Rhodesia, in 'Entomolgoical studies'. Publ.S.Afr.Inst.Med.Res. 7, 306.

Depinay, J. M., C. M. Mbogo, G. Killeen, B. Knols, J. Beier, J. Carlson, J. Dushoff, P. Billingsley, H. Mwambi, J. Githure, A. M. Toure, and F. E. McKenzie**.** 2004**.** A simulation model of African Anopheles ecology and population dynamics for the analysis of malaria transmission. *Malar J.* 3:29.: 29.

Devine, G. J. and I. Denhom**.** 1998. An unconventional use of piperonyl butoxide for managing the cotton whitefly, Bemisia tabci (Hemiptera: A byrodidae). Bulletin of Entomological Research. 88, 601-610.

Di Deco, M. A., N. Rishikeshi, V. Petrarca, and M. Coluzzi**.** 1981. Variazioni stagionali in *Anopheles gambiae* with *Anopheles arabiensis* at Kaduna, Nigeria. Parasitologia. 23, 169-172.

Dia, I., T. Diop, I. Rakotoarivony, P. Kengne, and D. Fontenille**.** 2003**.** Bionomics of *Anopheles gambiae* Giles, *An. arabiensis* Patton, An. funestus Giles and An. nili (Theobald) (Diptera: Culicidae) and transmission of Plasmodium falciparum in a Sudano-Guinean zone (Ngari, Senegal). *J Med Entomol.* 40(3): 279-283.

Diabate, A., T. Baldet, F. Chandre, R. T. Guiguemde, C. Brengues, P. Guillet, J. Hemingway, and J. M. Hougard**.** 2002**.** First report of the kdr mutation in *Anopheles gambiae* M form from Burkina Faso, west Africa. *Parassitologia.* 44(3-4): 157-158.

Diabate, A., F. Chandre, M. Rowland, R. N'guessan, S. Duchon, K. R. Dabire, and J. M. Hougard**.** 2006**.** The indoor use of plastic sheeting pre-impregnated with insecticide for control of malaria vectors. *Trop Med Int Health.* 11(5): 597-603.

Dietz, K. 1993.The estimation of the basic reproduction number for infectious diseases. Statistical Methods in Medical Research. 2, 23-41.

Diuk-Wasser, M. A., M. B. Toure, G. Dolo, M. Bagayoko, N. Sogoba, S. F. Traore, N. Manoukis, and C. E. Taylor. 2005. Vector abundance and malaria transmission in rice-growing villages in Mali. *Am. J. Trop. Med. Hyg.* 72(6): 725-731.

Dossou-Yovo, J., S. Diarrassouba, J. Doannio, F. Darriet, and P. Carnevale. 1999. [The aggressive cycle of *Anopheles gambiae s.s.* inside houses and malaria transmission in the Bouake region (Cote d'Ivoire). Value of using impregnated mosquito nets]. Bull Soc Pathol Exot. 92, 198-200.

Drakeley, C., R. Gosling, and H. Reyburn. 2005. Malaria diagnosis and treatment: one size does not fit all. *PLoS. Med.* 2(6): e156.

Du, W., T. S. Awolola, P. Howell, L. L. Koekemoer, B. D. Brooke, M. Q. Benedict, M. Coetzee, and L. Zheng. 2005. Independent mutations in the Rdl locus confer dieldrin resistance to Anopheles gambiae and An. arabiensis. *Insect Mol. Biol.* 14(2): 179-183.

ECZ. 2000. Environmental Council of Zambia, State of the Environment, Zambia Agricultural data-Final draft, December 2002. The IDL group. .

Ejov, M. N., T. Tun, S. Aung, S. Lwin, and K. Sein. 1999. Hospital-based study of severe malaria and associated deaths in Myanmar. Bull WorldHealth Organ. 77, 310-314.

Eliades, M. J., A. Wolkon, K. Morgah, S. B. Crawford, A. Dorkenoo, Y. Sodahlon, W. A. Hawley, A. W. Hightower, F. O. Kuile, and D. J. Terlouw. 2006. Burden of malaria at community level in children less than 5 years of age in Togo. *Am J Trop Med Hyg.* 75(4): 622-629.

Elissa, N., J. Mouchet, F. Riviere, J. Y. Meunier, and K. Yao. 1993. Resistance of *Anopheles gambiae* s.s. to pyrethroids in Cote D'Ivoire. Ann.Soc.Belg.Med.Trop. 73, 291-294.

Enayati, A. A., H. Ranson, and J. Hemingway. 2005. Mini Review: Insect glutathione transferases and Insecticide resistance. Insect Molecular Biology 14, 3-8.

Etang, J., E. Fondjo, F. Chandre, I. Morlais, C. Brengues, P. Nwane, M. Chouaibou, H. Ndjemai, and F. Simard. 2006. First report of knockdown mutations in the malaria vector *Anopheles gambiae* from Cameroon. *Am. J. Trop. Med. Hyg.* 74(5): 795-797.

Eto, M. 1974. Organophosphorus insecticides: organic and biological chemistry. CCR, Cleveland.

Fanshawe. 1971. The vegetation of Zambia.

Favia, G., G. Dimopoulos, A. Della Torre, Y. T. Toure, M. Coluzzi, and C. Louis. 1994. Polymorphisms detected by random PCR distinguishes between different chromosomal forms of *Anopheles gambiae*. Proc.Natl.Acad.Sci. 91, 10315-10319.

Faye, O., D. Fontenille, J. P. Herve, P. A. Diack, S. Diallo, and J. Mouchet. 1993. [Malaria in the Saharan region of Senegal. 1. Entomological transmission findings].

Ann. Soc. Belg. Med. Trop. 73(1): 21-30.

Feachem, R. and O. Sabot**.** 2008**.** A new global malaria eradication strategy. *Lancet.* 371(9624): 1633-1635.

Feachem, R. G. and O. J. Sabot**.** 2007**.** Global malaria control in the 21st century: a historic but fleeting opportunity. *JAMA.* 297(20): 2281-2284.

Feachem, R. G. A., A. A. Phillips, and G. A. Targett**.** 2009. Shrinking the malaria map. A prospectus on malaria elimination**.** San Francisco: The Global Health Group.

Fegan, G. W., A. M. Noor, W. S. Akhwale, S. Cousens, and R. W. Snow**.** 2007**.** Effect of expanded insecticide-treated bednet coverage on child survival in rural Kenya: a longitudinal study. *Lancet.* 370(9592): 1035-1039.

ffrench-Constant, R. H., J. C. Steichen, and F. Shotkoski**.** 1994. Polymerase chain reaction diagnostic for cyclodiene insecticide resistance in the mosquito *Aedes aegypti*. Med Vet Entomol 8, 99-100.

Fillinger, U., K. Kannady, G. William, M. J. Vanek, S. Dongus, D. Nyika, Y. Geissbuhler, P. P. Chaki, N. J. Govella, E. M. Mathenge, B. H. Singer, H. Mshinda, S. W. Lindsay, M. Tanner, D. Mtasiwa, M. C. De Castro, and G. F. Killeen**.** 2008**.** A tool box for operational mosquito larval control: preliminary results and early lessons from the Urban Malaria Control Programme in Dar es Salaam, Tanzania. *Malar. J.* 7:20.: 20.

Fillinger, U., G. Sonye, G. F. Killeen, B. G. Knols, and N. Becker**.** 2004**.** The practical importance of permanent and semipermanent habitats for controlling aquatic stages of *Anopheles gambiae* sensu lato mosquitoes: operational observations from a rural town in western Kenya. *Trop Med Int Health.* 9(12): 1274-1289.

Fontenille, D. and F. Simard**.** 2004.Unravelling complexities in human malaria transmission dynamics in Africa through a comprehensive knowledge of vector populations**.** Comp Immun Microbiol Infect Dis. 27, 357-375.

Fornadel, C. M., L. C. Norris, G. E. Glass, and D. E. Norris**.** 2010**.** Analysis of Anopheles arabiensis blood feeding behavior in southern Zambia during the two years after introduction of insecticide-treated bed nets. *Am J Trop Med Hyg.* 83(4): 848-853.

Foster, W. A. and E. D. Walker**.** 2002. Mosquitoes (*Culicidae*). In Mullen G, Durden L, eds, *Medical and Veterinary Entomology*. Academic Press, San Deigo.

Gabaldon, A. Malaria eradication in Venezuela: doctrine, practice, and achievements after twenty years. Am J Trop Med Hyg. 32, 203-211. 1983.

Gamble, C., J. P. Ekwaru, and F. O. ter Kuile**.** 2006**.** Insecticide-treated nets for preventing malaria in pregnancy. *Cochrane. Database. Syst. Rev.*(2): CD003755.

Garret-Jones, C. 1964. The human blood index of malaria vectors in relation to epidemiological assessment. Bulletin of the World Health Organization 30, 241-261.

Garrett-Jones, C. and G. R. Shidrawi**.** 1969**.** Malaria vectorial capacity of a population of Anopheles gambiae: an exercise in epidemiological entomology. *Bull World Health Organ.* 40(4): 531-545.

Geissbuhler, Y., P. Chaki, B. Emidi, N. J. Govella, R. Shirima, V. Mayagaya, D. Mtasiwa, H. Mshinda, U. Fillinger, S. W. Lindsay, K. Kannady, M. C. De Castro, M. Tanner, and G. F. Killeen**.** 2007**.** Interdependence of domestic malaria prevention measures and mosquito-human interactions in urban Dar es Salaam, Tanzania. *Malar J.* 6:126.: 126.

Georghiou, G. P. 1994.Principles of insecticide resistance management. Phytoprotection. 75, 51-59.

Georghiou, G. P. 1980. Insecticide resistance and prospects for its management. Residue Reviews. 76, 131-144.

Gething, P. W., A. M. Noor, P. W. Gikandi, E. A. Ogara, S. I. Hay, M. S. Nixon, R. W. Snow, and P. M. Atkinson**.** 2006**.** Improving imperfect data from health management information systems in Africa using space-time geostatistics. *PLoS. Med.* 3(6): e271.

Giglioli, G., C. Wan-I, P. Howell, and D. Marchant**.** 1974. Malaria eradication under continental equatorial conditions in Guyana. West Indian Med J. 23, 25-34.

Gilbert, S. C. and A. V. Hill**.** 1998**.** The SPf66 Malaria Vaccine: Where Do We Go From Here? *Parasitol Today.* 14(6): 251.

Gilles, H. M. and D. A. Warrell**.** 1993. Bruce-Chwatt's essential malariology, 3rd ed. London: Edward Arnold, pp. 129-136.

Gillies, M. T. and B. A. De Meillon**.** 1968. *The Anophelinae of Africa south of the Sahara (Ethiopian Zoogeographical Region)*. 2nd edn. South African Institute for Medical Research, Johannesburg. Publication of the South African Institute for Medical Research. 54, 131-132.

Gillies, M. T. 1988. Anopheline mosquitos: vector behaviour and bionomics. Edinburgh:Churchill Livingstone. 453-485.

Gillies, M. T. and M. Coetzee**.** 1987. *A supplement to the Anophelinae of Africa South of the Sahara (Afro-tropical region)*. Publication of the South African Institute for Medical Research, Johannesburg. 55, 78-143.

Gimnig, J. E., M. Ombok, L. Kamau, and W. A. Hawley**.** 2001. Characteristics of larval anopheline (Diptera: Culicidae) habitats in Western Kenya. J Med Entomol. 38, 282-288.

Gimnig, J. E., M. S. Kolczak, A. W. Hightower, J. M. Vulule, E. Schoute, L. Kamau, P. A. Phillips-Howard, F. O. ter Kuile, B. L. Nahlen, and W. A. Hawley**.** 2003**.** Effect of permethrin-treated bed nets on the spatial distribution of malaria vectors in western Kenya. *Am J Trop Med Hyg.* 68(4 Suppl): 115-120.

Githeko, A. K. 2006.Entomological correlates of epidemiological impacts:how do

we know its working. In Knows, B.G.J and Louis, C (Eds) Bridging Laboratory and Field Research for Genetic Control of Disease Vectors. Wageningen UR Frontis Series 11, 215-219.

Goodman, C. A., P. A. Coleman, and A. J. Mills. 1999. Cost effectiveness of malaria control in sub-Saharan Africa. Lancet 354, 378-385.

Goodman, C. A., A. E. Mnzava, S. S. Dlamini, B. L. Sharp, D. J. Mthembu, and J. K. Gumede. 2001. Comparison of the cost and cost-effectiveness of insecticide-treated bednets and residual house-spraying in KwaZulu-Natal, South Africa. *Trop. Med. Int. Health.* 6(4): 280-295.

Grant, D. F. and B. D. Hammock. 1992.Genetic and molecular evidence for a trans-acting regulatory locus controlling glutathione S-transferase-2 expression in Aedes aegypti. Molecular andGeneral Genetics. 234, 169-176.

Grant, D. F. and F. Matsumura. 1989.Glutathione S-transferase 1 and 2 in susceptible and insecticide resistant *Aedes aegypti.* Pestic.Biochem.Physiol. 33, 132-143.

Graves, P. M., D. E. Osgood, M. C. Thomson, K. Sereke, A. Araia, M. Zerom, P. Ceccato, M. Bell, C. J. Del, S. Ghebreselassie, E. P. Brantly, and T. Ghebremeskel. 2008. Effectiveness of malaria control during changing climate conditions in Eritrea, 1998-2003. *Trop Med Int Health.* 13(2): 218-228.

Greenberg, A. E., M. Ntumbanzondo, N. Ntula, L. Mawa, J. Howell, and F. Davachi. 1989. Hospital-based surveillance of malaria-related paediatric morbidity and mortality in Kinshasa, Zaire. *Bull World Health Organ.* 67(2): 189-196.

Greenwood, B. and T. Mutabingwa. 2002. Malaria in 2002. *Nature.* 415(6872): 670-672.

Greenwood, B. M. 2008. Control to elimination: implications for malaria research. *Trends Parasitol.* 24(10): 449-454.

Greever, J. and G. P. Georghiou. 1979.Computer simulation of control strategies for *Culex tarsalis* (Diptera: Culicidae). Journal of Medical Entomology. 16, 180-188.

Gregory, R. D. and T. M. Blackburn. 1991.Parasite prevalence and host sample size. Parasitol Today. 7, 316-318.

Guerra, C. A., S. I. Hay, L. S. Lucioparedes, P. W. Gikandi, A. J. Tatem, A. M. Noor, and R. W. Snow. 2007. Assembling a global database of malaria parasite prevalence for the Malaria Atlas Project. *Malar. J.* 6:17.: 17.

Guerra, C. A., R. W. Snow, and S. I. Hay. 2006. Mapping the global extent of malaria in 2005. *Trends Parasitol.* 22(8): 353-358.

Guessan, R. N., V. Corbel, M. Akogbéto, and M. Rowland. 2007.Reduced Efficacy of Insecticide treated Nets and Indoor Residual Spraying for Malaria Control in Pyrethroid Resistance Area, Benin. Emerging Infectious Diseases. 13, 199-206.

Guyatt, H. L., J. Kinnear, M. Burini, and R. W. Snow. 2002. A comparative cost analysis of insecticide-treated nets and indoor residual spraying in highland Kenya. *Health Policy Plan.* 17(2): 144-153.

Hackett, L. W. 1937. Malaria in Europe. Oxford, Oxford University Press.

Haddow, A. J. 1943. Measurement of temperature and light in artificial pools with reference to the larval habitat of *Anopheles (Myzomia) gambiae* Giles and *A. (M.) funestus* Giles. Bull Entomol Res. 34, 89.

Hamon, J. and J. Mouchet. 1961.Les vetours secondaires du paludisme humain en Afrique. Medecine Tropicale 221, 643-660.

Hargreaves, K., L. L. Koekemoer, B. D. Brooke, R. H. Hunt, J. Mthembu, and M. Coetzee. 2000. *Anopheles funestus* resistant to pyrethroid insecticides in South Africa. *Med. Vet. Entomol.* 14(2): 181-189.

Hassal, K. 1990.The biochemistry and uses of pesticides. 2nd. Edition. Macmillan Press LTD.

Hawley, W. A., F. O. ter Kuile, R. S. Steketee, B. L. Nahlen, D. J. Terlouw, J. E. Gimnig, Y. P. Shi, J. M. Vulule, J. A. Alaii, A. W. Hightower, M. S. Kolczak, S. K. Kariuki, and P. A. Phillips-Howard. 2003. Implications of the western Kenya permethrin-treated bed net study for policy, program implementation, and future research. *Am J Trop Med Hyg.* 68(4 Suppl): 168-173.

Hay, S. I., C. J. Tucker, D. J. Rogers, and M. J. Packer. Remotely sensed surrogates of meteorological data for the study of the distribution and abundance of arthropod vectors of disease. Ann Trop Med Parasitol. 90, 1-19. 1996.
Ref Type: Generic

Hay, S. I., C. A. Guerra, A. J. Tatem, A. M. Noor, and R. W. Snow. 2004. The global distribution and population at risk of malaria: past, present, and future. *Lancet Infect. Dis.* 4(6): 327-336.

Hay, S. I., J. A. Omumbo, M. H. Craig, and R. W. Snow. 2000. Earth observation, geographic information systems and *Plasmodium falciparum* malaria in sub-Saharan Africa. *Adv. Parasitol.* 47:173-215.: 173-215.

Hay, S. I., D. L. Smith, and R. W. Snow. 2008. Measuring malaria endemicity from intense to interrupted transmission. *Lancet Infect Dis.* 8(6): 369-378.

Hayes, J. D. and D. J. Pulford. 1995. The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit Rev . Biochem Mol Biol. 30, 445-600.

Hays, C. W. 2000.The United States Army and malaria control in World War II. Parassitologia. 42, 47-52.

Hemingway, J. 1989.A note on simple biochemical methods for resistance detection and their field application in Sri Lanka. Pesticide Science. 27, 281-285.

Hemingway, J., B. J. Beaty, M. Rowland, T. W. Scott, and B. L. Sharp**.** 2006.The Innovative Vector Control Consortium: Improved control of mosquito-borne diseases. Trends Parasitol. 22, 308-312.

Hemingway, J. and G. P. Georghiou**.** 1983.Studies on the acetylcholinesterase of *Anopheles albimanus* resistant and susceptible to organophosphate and carbamate insecticides. Pestic.Biochem.Physiol. 19, 167-171.

Hemingway, J. and S. H. P. P. Karunaratne**.** 1998. Mosquito carboxylesterases: a review of the molecular biology and biochemistry of a major insecticide resistance mechanism. Med.Vet.Entomol. 12, 1-12.

Hemingway, J., C. A. Malcolm, K. E. Kissoon, R. G. Boddington, C. F. Curtis, and N. Hill**.** 1985.The biochemistry of insecticide resistance in *Anopheles sacharovi*: comparative studies with a range of insecticide susceptible and resistant *Anopheles* and *Culex* species. Pestic.Biochem.Physiol. 24, 68-76.

Hemingway, J., C. Smith, K. J. I. Jayawarden, and P. R. J. earth**.** 1986.Field and laboratory detection of the altered acetylcholinesterase resistance genes which confer organophosphate and carbamate resistance in mosquitoes (Diptera: Culicidae). Bulletin of Entomological Reserch 76, 559-565.

Hemingway, J. 1981.Genetics and Biochemistry of insecticide resistance in Anophelines.Ph.D. thesis. London University.

Hemingway, J. 1982.The biochemical nature of malathion resistance in *Anopheles stephensi* from Pakistan. Pestic.Biochem.Physiol. 17, 149-155.

Hemingway, J. 1983.Biochemical studies on malathion resistance in *Anopheles arabiensis* from Sudan. Trans.R.Soc.Trop.Med.Hyg. 77, 477-480.

Hemingway, J. 1985.Malathion carboxylesterase enzymes in *Anopheles arabiensis* from Sudan. Pest.Biochem.Physiol. 23, 309-313.

Hemingway, J. 2004**.** Taking aim at mosquitoes. *Nature.* 430(7002): 936.

Hemingway, J. and I. Bates**.** 2003**.** Malaria: past problems and future prospects. After more than a decade of neglect, malaria is finally black on the agenda for both biomedical research and public health politics. *EMBO Rep.* 4 Spec No:S29-31.: S29-S31.

Hemingway, J., L. Field, and J. Vontas**.** 2002**.** An overview of insecticide resistance. *Science.* 298(5591): 96-97.

Hemingway, J., R. P. Penilla, A. D. Rodriguezi, B. M. James, W. Edge, H. Rogers, and M. H. Rodriguez**.** 1997.Resistance management strategies in malaria vector mosquito control. A large scale trial in southern Mexico. Pesticide Science. 51, 375-382.

Hemingway, J. and H. Ranson**.** 2000.Insecticide resistance in insect vectors of human disease. Annual Review of Entomology. 45, 371-391.

Hemingway, J., G. J. Small, S. Lindsay, and F. H. Collins. 1995.Combined use of biochemical, immunological and molecular assays for infection, species identification and resistance detection in field populations of *Anopheles* (Diptera: Culicidae). The Ecology ofAgricultural Pests: Biochemical Approaches (ed.by W.O.C.Symondson and E.Lydell).Chapman and Hall, London. 31-49.

Henry, M. C., S. B. Assi, C. Rogier, J. Dossou-Yovo, F. Chandre, P. Guillet, and P. Carnevale. 2005. Protective efficacy of lambda-cyhalothrin treated nets in *Anopheles gambiae* pyrethroid resistance areas of Cote d'Ivoire. *Am J Trop Med Hyg.* 73(5): 859-864.

Herath, P. R. and G. P. Joshi. 1986. Factors affecting selection for multiple resistance in Anopheles nigerrimus in Sri Lanka. *Trans R Soc Trop Med Hyg.* 80(4): 649-652.

Herath, P. R. J. and G. Davidson. 1981. Multiple resistance in *Anopheles culicifacies* Giles. Mosq.News 41, 325-327.

Herath, P. R. J., S. J. Miles, and G. Davidson. 1981. Fenitrothion (OMS 43) resistance in the taxon *Anopheles culicifacies* Giles. J.Trop.Med.Hyg. 84, 87-88.

Hill, A. V. 2006. Aspects of genetic susceptibility to human infectious diseases. *Annu Rev Genet.* 40:469-86.: 469-486.

Himpoo, B. and J. B. MacCallum. 1967. Possible chloroquine resistant mal-aria in Zambia. Med.J.Zambia. 1, 31-35.

Hoshen, M. B. and A. P. Morse. 2004. A weather-driven model of malaria transmission. *Malar. J.* 3:32.: 32.

Hoskins, W. M. and R. Craig. 1962. Uses of bioassay in entomology. Annual.Rev.Entomol. 7, 437-464.

Hunt, R. H., M. Coetzee, and Messay Fettene. 1998. The *Anopheles gambiae* complex: a new species from Ethiopia. Transactions of the Royal Society of Tropical Medicine and Hygiene. 92, 231-235.

Ijumba, J. and S. Lindsay. 2001. Impact of irrigation on malaria in Africa: paddies paradox. Med Vet Entomol. 15, 1-11.

Ilboudo-Sanogo, E., N. Cuzin-Ouattara, D. A. Diallo, S. N. Cousens, F. Esposito, A. Habluetzel, S. Sanon, and A. P. Ouedraogo. 2001. Insecticide-treated materials, mosquito adaptation and mass effect: entomological observations after five years of vector control in Burkina Faso. *Trans. R. Soc. Trop. Med. Hyg.* 95(4): 353-360.

Immaraju, J. A., J. G. Morse, and R. F. Hobza. 1990. Field evaluation of insecticide rotation and mixtures as strategies for citrus thrips (Thysanoptera; Thripidae) resistance management in California. CSA. J.Econ.Entomol. 83, 306-314.

IRAC. 2006. *Prevention and management of insecticide resistance in vectors and pests of public health importance*. Monograph, 51pp.

Jovani, R. and J. L. Tella. 2006. Parasite prevalence and sample size: misconceptions and solutions. TrendsParasitol 22, 214-218.

Kadous, A. A., S. M. Ghiasuddin, F. Matsumura, J. G. Scott, and K. Tanaka. 1983. Difference in the picrotoxinin receptor between the cyclodiene-resistant and susceptible strains of the German cockroach. Pest.Biochem.Physiol. 19, 157-166.

Karch, S., B. Garin, N. Asidi, Z. Manzambi, and J. J. e. al. Salaun. 1993. [Mosquito nets impregnated against malaria in Zaire]. Ann Soc Belg Med Trop. 73, 37-53.

Kasai, S., I. S. Weerasinghe, and T. Shono. 1998. P450 Monooxygenases are an important mechanism of permethrin resistance in *Culex quinquefasciatus* say larvae. Arch.Insect Biochem.Physiol. 37, 47-56.

Kazembe, L. N., I. Kleinschmidt, and B. L. Sharp. 2006. Patterns of malaria-related hospital admissions and mortality among Malawian children: an example of spatial modelling of hospital register data. *Malar J.* 5:93.: 93.

Keating, J., K. Macintyre, C. Mbogo, A. Githeko, J. L. Regens, C. Swalm, B. Ndenga, L. J. Steinberg, L. Kibe, J. I. Githure, and J. C. Beier. 2003. A geographic sampling strategy for studying relationships between human activity and malaria vectors in urban Africa. *Am. J. Trop. Med. Hyg.* 68(3): 357-365.

Keating, J., J. M. Miller, A. Bennett, H. B. Moonga, and T. P. Eisele. 2009. *Plasmodium falciparum* parasite infection prevalence from a household survey in Zambia using microscopy and a rapid diagnostic test: implications for monitoring and evaluation. *Acta Trop.* 112(3): 277-282.

Keiser, J., B. H. Singer, and J. Utzinger. 2005. Reducing the burden of malaria in different eco-epidemiological settings with environmental management: a systematic review. *Lancet Infect. Dis.* 5(11): 695-708.

Kelly-Hope, L., H. Ranson, and J. Hemingway. 2008. Lessons from the past: managing insecticide resistance in malaria control and eradication programmes. *Lancet Infect Dis.* 8(6): 387-389.

Kengne, P., P. Awono-Ambene, C. Antonio-Nkondjio, F. Simard, and D. Fontenille. 2003. Molecular identification of the *Anopheles nili* group of African malaria vectors. *Med Vet. Entomol.* 17(1): 67-74.

Kent, R. J., S. Mharakurwa, and D. E. Norris. 2007. Spatial and temporal genetic structure of *Anopheles arabiensis* in Southern Zambia over consecutive wet and drought years. *Am. J. Trop. Med. Hyg.* 77(2): 316-323.

Kent, R. J., P. E. Thuma, S. Mharakurwa, and D. E. Norris. 2007. Seasonality, blood feeding behavior, and transmission of *Plasmodium falciparum* by *Anopheles arabiensis* after an extended drought in southern Zambia. *Am. J. Trop. Med. Hyg.* 76(2): 267-274.

Killeen, G. F., B. G. Knols, U. Fillinger, J. C. Beier, and L. C. Gouagna. 2002. Interdisciplinary malaria vector research and training for Africa. *Trends Parasitol.* 18(10): 433-434.

Killeen, G. F., F. E. McKenzie, B. D. Foy, C. Bogh, and J. C. Beier**.** 2001**.** The availability of potential hosts as a determinant of feeding behaviours and malaria transmission by African mosquito populations. *Trans. R. Soc. Trop. Med. Hyg.* 95(5): 469-476.

Killeen, G. F., F. E. McKenzie, B. D. Foy, C. Schieffelin, P. F. Billingsley, and J. C. Beier**.** 2000**.** A simplified model for predicting malaria entomologic inoculation rates based on entomologic and parasitologic parameters relevant to control. *Am. J. Trop. Med. Hyg.* 62(5): 535-544.

Killeen, G. F., A. Seyoum, and B. G. Knols**.** 2004**.** Rationalizing historical successes of malaria control in Africa in terms of mosquito resource availability management. *Am. J. Trop. Med. Hyg.* 71(2 Suppl): 87-93.

Killeen, G. F. and T. A. Smith**.** 2007**.** Exploring the contributions of bed nets, cattle, insecticides and excitorepellency to malaria control: a deterministic model of mosquito host-seeking behaviour and mortality. *Trans. R. Soc Trop Med Hyg.* 101(9): 867-880.

Killeen, G. F., T. A. Smith, H. M. Ferguson, H. Mshinda, S. Abdulla, C. Lengeler, and S. P. Kachur**.** 2007**.** Preventing childhood malaria in Africa by protecting adults from mosquitoes with insecticide-treated nets. *PLoS. Med.* 4(7): e229.

Killeen, G. F., M. Tanner, W. R. Mukabana, M. S. Kalongolela, K. Kannady, S. W. Lindsay, U. Fillinger, and M. C. De Castro**.** 2006**.** Habitat targeting for controlling aquatic stages of malaria vectors in Africa. *Am. J. Trop. Med. Hyg.* 74(4): 517-518.

Kirby, M. J. and S. W. Lindsay**.** Responses of adult mosquitoes of two sibling species,*Anopheles arabiensis* and *A. gambiae s.s.* (Diptera: Culicidae), to high temperatures. Bull Entomol Res. 94, 441-448. 2004.
Ref Type: Generic

Kiszewski, A., A. Mellinger, A. Spielman, P. Malaney, S. E. Sachs, and J. Sachs**.** 2004**.** A global index representing the stability of malaria transmission. *Am J Trop Med Hyg.* 70(5): 486-498.

Kitron, U. and A. Spielman**.** 1989**.** Suppression of transmission of malaria through source reduction: antianopheline measures applied in Israel, the United States, and Italy. *Rev. Infect. Dis.* 11(3): 391-406.

Kleinschmidt, I., C. Schwabe, M. Shiva, J. L. Segura, V. Sima, S. J. Mabunda, and M. Coleman**.** 2009**.** Combining indoor residual spraying and insecticide-treated net interventions. *Am. J. Trop. Med. Hyg.* 81(3): 519-524.

Kleinschmidt, I. and B. Sharp**.** 2001**.** Patterns in age-specific malaria incidence in a population exposed to low levels of malaria transmission intensity. *Trop. Med. Int. Health.* 6(12): 986-991.

Kleinschmidt, I., B. Sharp, L. E. Benavente, C. Schwabe, M. Torrez, J. Kuklinski, N. Morris, J. Raman, and J. Carter**.** 2006**.** Reduction in infection with *Plasmodium falciparum* one year after the introduction of malaria control interventions on Bioko Island, Equatorial Guinea. *Am. J. Trop. Med. Hyg.* 74(6): 972-978.

Kleinschmidt, I., M. Torrez, C. Schwabe, L. Benavente, I. Seocharan, D. Jituboh, G. Nseng, and B. Sharp**.** 2007**.** Factors influencing the effectiveness of malaria control in Bioko Island, equatorial Guinea. *Am. J. Trop. Med. Hyg.* 76(6): 1027-1032.

Klinkenberg, E., P. J. McCall, M. D. Wilson, A. O. Akoto, F. P. Amerasinghe, I. Bates, F. H. Verhoeff, G. Barnish, and M. J. Donnelly**.** 2006**.** Urban malaria and anaemia in children: a cross-sectional survey in two cities of Ghana. *Trop. Med. Int. Health.* 11(5): 578-588.

Koekemoer, L. L., L. Kamau, R. H. Hunt, and M. Coetzee**.** 2002**.** A cocktail polymerase chain reaction assay to identify members of the *Anopheles funestus* (Diptera: Culicidae) group. *Am. J. Trop. Med. Hyg.* 66(6): 804-811.

Kofi Ekue, J. M. K., A.-M. Ulrich, and E. K. Njelesani**.** 1983. Plasmodium malaria resistant to chloroquine in a Zambian living in Zambia. Brit.med.J. 286, 1315-1316.

Kolaczinski, J., K. Graham, A. Fahim, S. Brooker, and M. Rowland**.** 2005**.** Malaria control in Afghanistan: progress and challenges. *Lancet.* 365(9469): 1506-1512.

Komatsu, R., E. L. Korenromp, D. Low-Beer, C. Watt, C. Dye, R. W. Steketee, B. L. Nahlen, R. Lyerla, J. M. Garcia-Calleja, J. Cutler, and B. Schwartlander**.** 2010**.** Lives saved by Global Fund-supported HIV/AIDS, tuberculosis and malaria programs: estimation approach and results between 2003 and end-2007. *BMC. Infect. Dis.* 10:109.: 109.

Komatsu, R., D. Low-Beer, and B. Schwartlander**.** 2007**.** Global Fund-supported programmes contribution to international targets and the Millennium Development Goals: an initial analysis. *Bull. World Health Organ.* 85(10): 805-811.

Korenromp, E. L., J. R. rmstrong-Schellenberg, B. G. Williams, B. L. Nahlen, and Snow.R.W. 2004. Impact of malaria control on childhood anaemia in Africa -- a quantitative review. Trop.Med.Int.Health. 9, 1050-1065.

Kotze, A. C. and N. Sales**.** 2001**.** Inheritance of diflubenzuron resistance and monooxygenase activities in a laboratory-selected strain of Lucilia cuprina (Diptera: Calliphoridae). *J Econ. Entomol.* 94(5): 1243-1248.

Kouznetsov, R. L. 1977**.** Malaria control by application of indoor spraying of residual insecticides in tropical Africa and its impact on community health. *Trop Doct.* 7(2): 81-91.

Krafsur, E. S. 1970**.** *Anopheles nili* as a vector of malaria in a lowland region of Ethiopia. *Bull. World Health Organ.* 42(3): 466-471.

Kulkarni, M. A., E. Kweka, E. Nyale, E. Lyatuu, F. Mosha, and et al. 2006. Entomological evaluation of malaria vectors at different altitudes in Hai district, northeastern Tanzania. J Med Entomol. 43, 580-588.

Lagunes Tejeda, A. 1980. Impact of the Use of Mixtures and Sequences of Insecticides in the Evolution of Resistance in Culex quinquefasciatus Say (Diptera: Culicidae). *Ph.D.Thesis, Univ.of California, Riverside, CA* 229 p.

Le Menach, A., F. E. McKenzie, A. Flahault, and D. L. Smith. 2005. The unexpected importance of mosquito oviposition behaviour for malaria: non-productive larval habitats can be sources for malaria transmission. *Malar. J.* 4(1): 23.

Le Menach, A., S. Takala, F. E. McKenzie, A. Perisse, A. Harris, A. Flahault, and D. L. Smith. 2007. An elaborated feeding cycle model for reductions in vectorial capacity of night-biting mosquitoes by insecticide-treated nets. *Malar. J.* 6:10.: 10.

Lehmann, T., W. A. Hawley, H. Grebert, and et al. 1999. The rift valley complex as a barrier to gene flow for *Anopheles gambiae* in Kenya. Journal of Heredity. 90, 613-621.

Lehmann, T., M. Licht, N. Elissa, B. T. Maega, J. M. Chimumbwa, F. T. Watsenga, C. S. Wondji, F. Simard, and W. A. Hawley. 2003. Population Structure of Anopheles gambiae in Africa. *J. Hered.* 94(2): 133-147.

Lengeler, C. and B. Sharp. 2003. Indoor residual spraying and insecticide-treated nets, In Reducing Malaria's Burden: Evidence of Effectiveness for Decision Makers. Global Health Council Technical Report - December 2003 pp17-24.

Lengeler, C. 2004. Insecticide-treated bed nets and curtains for preventing malaria. *Cochrane. Database. Syst. Rev.*(2): CD000363.

Lengeler, C. 2004. Insecticide-treated nets for malaria control: real gains. *Bull. World Health Organ.* 82(2): 84.

Lengeler, C., J. Armstrong-Schellenberg, U. D'Alessandro, F. Binka, and J. Cattani. 1998. Relative versus absolute risk of dying reduction after using insecticide-treated nets for malaria control in Africa. *Trop Med Int Health.* 3(4): 286-290.

Lengeler, C., M. Grabowsky, D. McGuire, and D. deSavigny. 2007. Quick wins versus sustainability: options for the upscaling of insecticide-treated nets. *Am J Trop Med Hyg.* 77(6 Suppl): 222-226.

Lengeler, C., J. A. Schellenberg, and U. D'Alessandro. 1995. Will reducing Plasmodium falciparum malaria transmission alter malaria mortality among African children? *Parasitol Today.* 11(11): 425.

Lengeler, C., T. A. Smith, and S. J. Armstrong. 1997. Focus on the effect of bednets on malaria morbidity and mortality. *Parasitol Today.* 13(3): 123-124.

Lenormand, T., D. Bourguet, T. Guillemaud, and M. Raymond. 1999. Tracking the evolution of insecticide resistance in the mosquito *Culex pipiens*. Nature 400, 861-864.

Lindblade, K. A., E. D. Walker, A. W. Onapa, J. Katungu, and M. L. Wilson. 1999. Highland malaria in Uganda; Prospective analysis of an epidemic associated with El Nino. Transactions for Royal Society of Tropical Medicine and Hygiene. 93, 480-487.

Lindblade, K. A., E. D. Walker, and M. L. Wilson. 2000. Early warning of malaria

epidemics in African highlands using Anopheles (Diptera: Culicidae) indoor resting density. *J. Med. Entomol.* 37(5): 664-674.

Lindsay, S., J. Ansell, C. Selman, and et al. 2000. Effect of pregnancy on exposure to malaria mosquitoes. Lancet. 355, 1972.

Lindsay, S. W., J. R. M. Armstrong Schellenberg, H. A. Zeiler, R. J. Daly, and F. M. e. al. Salum**.** 1995.Exposure of Gambian children to *Anopheles gambiae* vectors in an irrigated rice production area. Med Vet Entomol. 9, 50-58.

Lindsay, S. W., P. M. Emerson, and J. D. Charlwood**.** 2002. Reducing malaria transmission by mosquito-proofing homes. Trends Parasitol. 18, 510-514.

Lindsay, S. W., J. H. Adiamah, J. E. Miller, R. J. Pleass, and J. R. Armstrong**.** 1993**.** Variation in attractiveness of human subjects to malaria mosquitoes (Diptera: Culicidae) in The Gambia. *J. Med. Entomol.* 30(2): 368-373.

Lindsay, S. W. and M. H. Birley**.** 1996**.** Climate change and malaria transmission. *Ann Trop Med Parasitol.* 90(6): 573-588.

Lindsay, S. W. and W. J. Martens**.** 1998**.** Malaria in the African highlands: past, present and future. *Bull. World Health Organ.* 76(1): 33-45.

Lindsay, S. W., L. Parson, and C. J. and Thomas**.** 1998. Mapping the ranges and relative abundance of the two principal African malaria vectors, *Anopheles gambiae sensu stricto* and *An. arabiensis*, using climate data. Proceedings of the Royal Society of London.Series B.Biological sciences. 265, 847-854.

Lindsay, S. W., R. W. Snow, G. L. Broomfield, M. S. Janneh, R. A. Wirtz, and B. M. Greenwood**.** 1989**.** Impact of permethrin-treated bednets on malaria transmission by the Anopheles gambiae complex in The Gambia. *Med Vet Entomol.* 3(3): 263-271.

Lindsay, S. W., H. A. Wilkins, H. A. Zieler, R. J. Daly, V. Petrarca, and P. Byass**.** 1991**.** Ability of Anopheles gambiae mosquitoes to transmit malaria during the dry and wet seasons in an area of irrigated rice cultivation in The Gambia. *J. Trop. Med. Hyg.* 94(5): 313-324.

Lines, J., C. Lengeler, K. Cham, S. D. de, J. Chimumbwa, P. Langi, D. Carroll, A. Mills, K. Hanson, J. Webster, M. Lynch, W. Addington, J. Hill, M. Rowland, E. Worrall, M. MacDonald, and A. Kilian**.** 2003**.** Scaling-up and sustaining insecticide-treated net coverage. *Lancet Infect. Dis.* 3(8): 465-466.

Lines, J. D. 1988**.** Do agricultural insecticides select for insecticide resistance in mosquitoes? A look at the evidence. *Parasitol. Today.* 4(7): S17-S20.

Lines, J. D., J. Myamba, and C. F. Curtis**.** 1987**.** Experimental hut trials of permethrin-impregnated mosquito nets and eave curtains against malaria vectors in Tanzania. *Med Vet Entomol.* 1(1): 37-51.

Lynd, A., H. Ranson, P. J. McCall, N. P. Randle, W. C. Black, E. D. Walker, and M. J. Donnelly**.** 2005**.** A simplified high-throughput method for pyrethroid knock-down

resistance (kdr) detection in Anopheles gambiae. *Malar. J.* 4(1): 16.

Mabaso, M. L., B. Sharp, and C. Lengeler**.** 2004**.** Historical review of malarial control in southern African with emphasis on the use of indoor residual house-spraying. *Trop Med Int Health.* 9(8): 846-856.

Macdonald, G. 1957. *The epidemiology and control of malaria*. Oxford University. Press, London.
Machado, C. A. and J. Hey**.** 2003. The causes of phylogenetic conflict in a classic Drosophila species group. Proc R Soc Lond B Biol Sci. 270, 1193-1202.

Magesa, S. M., T. J. Wilkes, A. E. Mnzava, K. J. Njunwa, J. Myamba, M. D. Kivuyo, N. Hill, J. D. Lines, and C. F. Curtis**.** 1991**.** Trial of pyrethroid impregnated bednets in an area of Tanzania holoendemic for malaria. Part 2. Effects on the malaria vector population. *Acta Trop.* 49(2): 97-108.

Maharaj, R., D. J. Mthembu, and B. L. Sharp**.** 2005**.** Impact of DDT re-introduction on malaria transmission in KwaZulu-Natal. *S. Afr. Med. J.* 95(11): 871-874.

Malcolm, C. A. and R. G. Boddington**.** 1989. Malathion resistance conferred by a carboxylesterase in *Anopheles culicifacies* Giles (Species B) (Diptera: Culicidae). Bull.Entomol.Res. 79, 193-199.

Malcolm, C. A. 1988. Current status of pyrethroid resistance in Anophelines. Parasitology Today. 4, S13-S15.

Manda, H., L. C. Gouagna, W. A. Foster, R. R. Jackson, J. C. Beier, J. I. Githure, and A. Hassanali**.** 2007**.** Effect of discriminative plant-sugar feeding on the survival and fecundity of Anopheles gambiae. *Malar. J.* 6:113.: 113.

Mani, G. S. 1985. Evolution of resistance in the presence of two insecticides. Genetics. 109, 761-783.

Marinez-Ramirez, E. Dolores, J. Silva, and J. Ferree**.** 1995.Inheritance of resistance to a *Bacillus thuringesis* toxin in a field population of diamondback moth (*Plutella xylostella*). Pesticide Science. 43, 115-120.

Marsh, K. 1998**.** Malaria disaster in Africa. *Lancet.* 352(9132): 924.

Martens, P. and L. Hall**.** 2000**.** Malaria on the move: human population movement and malaria transmission. *Emerg. Infect. Dis.* 6(2): 103-109.

Martinez-Torres, D., F. Chandre, M. S. Williamson, F. Darriet, J. B. Berge, A. L. Devonshire, P. Guillet, N. Pasteur, and D. Pauron**.** 1998**.** Molecular characterization of pyrethroid knockdown resistance (kdr) in the major malaria vector Anopheles gambiae s.s. *Insect Mol. Biol.* 7(2): 179-184.

Matsumura, F. 1975. Toxicology of insecticides. Plenum Press, New York and London.

Maxwell, C. A., E. Msuya, M. Sudi, K. J. Njunwa, I. A. Carneiro, and C. F. Curtis**.** 2002**.** Effect of community-wide use of insecticide-treated nets for 3-4 years on

malarial morbidity in Tanzania. *Trop Med Int Health.* 7(12): 1003-1008.

May, R. 1985. Evolution of pesticide resistance. Nature 315, 12-13.

Mbogo, C. N. M., N. M. Baya, A. V. O. Ofulla, J. I. Githure, and R. W. Snow**.** 1996. The impact of permethrin-impregnated bednets on malaria vectors of the Kenyan coast. Med VetEntomol. 10, 251-259.

Mc Wilsons, W., P. Billing, D. Bendahmane, and P. and Wijeyanratne**.** 1999. Malaria in rural and peri-urban areas in Sub-Saharan Africa. *Environmental Health Project Activity Report* **71**.

McClean, K. L. and A. Senthilselvan**.** 2002**.** Mosquito bed nets: implementation in rural villages in Zambia and the effect on subclinical parasitaemia and haemoglobin. *Trop. Doct.* 32(3): 139-142.

McKenzie, F. E., J. Sirichaisinthop, R. S. Miller, R. A. Gasser, Jr., and C. Wongsrichanalai**.** 2003**.** Dependence of malaria detection and species diagnosis by microscopy on parasite density. *Am J Trop Med Hyg.* 69(4): 372-376.

McMichael, A. J. and A. Haines**.** 1997. Global climate change: the potential effects on health. BMJ 315, 805.

Mellon, R. B. and G. P. Georghiou**.** 1984. Rotational use of insecticides in mosquito control programmes. Proceeding of the 52nd Annual Conference of the California Mosquito and Vector ControlAssociation , 65-67.

Mendis, K., A. Rietveld, M. Warsame, A. Bosman, B. Greenwood, and W. H. Wernsdorfer**.** 2009**.** From malaria control to eradication: The WHO perspective. *Trop Med Int Health.* 14(7): 802-809.

Menendez, C., E. Kahigwa, R. Hirt, P. Vounatsou, J. J. Aponte, F. Font, C. J. Acosta, D. M. Schellenberg, C. M. Galindo, J. Kimario, H. Urassa, B. Brabin, T. A. Smith, A. Y. Kitua, M. Tanner, and P. L. Alonso**.** 1997**.** Randomised placebo-controlled trial of iron supplementation and malaria chemoprophylaxis for prevention of severe anaemia and malaria in Tanzanian infants. *Lancet.* 350(9081): 844-850.

Metselaar, D. and P. H. Van Thiel**.** 1959. Classification of malaria. Trop Geogr Med 11, 157-161.

Milani, R. 1963. Genetical aspects of insecticide resistance. Bulletin of the World Health Organ. 29, 77-97.

Miller, J. E., S. W. Lindsay, and J. R. Armstrong**.** 1991**.** Experimental hut trials of bednets impregnated with synthetic pyrethroid or organophosphate insecticide for mosquito control in The Gambia. *Med Vet Entomol.* 5(4): 465-476.

Miller, L. H. and S. L. Hoffman**.** 1998**.** Research toward vaccines against malaria. *Nat. Med.* 4(5 Suppl): 520-524.

Miller, T. A. and V. L. Salgado**.** 1985. The mode of action of pyrethroids on insects.

In: The pyrethroid insecticides. Leahey. J. P. [ed]. Taylor & Francis Publ, London & Philadelphia. pp. 440.

Minakawa, N., J. I. Githure, J. C. Beier, and G. Yan. 2001. Anopheline mosquito survival strategies during the dry period in western Kenya. J Med Entomol. 38, 388-392.

Minakawa, N., C. M. Mutero, J. I. Githure, J. C. Beier, and G. Yan. 1999. Spatial distribution and habitat characterization of Anopheline mosquito larvae in Western Kenya. Am J Trop MedHyg. 61, 1010-1016.

Minakawa, N., P. Seda, and G. Yan. 2002. Influence of host and larval habitat distribution on the abundance of African malaria vectors in Western Kenya. Am J Trop Med Hyg. 67, 32-38.

Minakawa, N., E. Omukunda, G. Zhou, A. Githeko, and G. Yan. 2006. Malaria vector productivity in relation to the highland environment in Kenya. *Am. J. Trop. Med. Hyg.* 75(3): 448-453.

Minakawa, N., G. Sonye, M. Mogi, and G. Yan. 2004. Habitat characteristics of Anopheles gambiae s.s. larvae in a Kenyan highland. *Med. Vet. Entomol.* 18(3): 301-305.

Miyazaki, M., K. Ohyama, D. Y. Dunlap, and F. Matsumura. 1996. Cloning and sequencing of the para-type sodium channel gene from susceptible and kdr-resistant German cockroaches (Blatella germanica) and house fly (Musca domestica). Mol Gen Genet. 252, 61-68.

Modiano, D., V. Petrarca, B. S. Sirima, I. Nebie, G. Luoni, F. Esposito, and M. Coluzzi. 1998. Baseline immunity of the population and impact of insecticide-treated curtains on malaria infection. *Am J Trop Med Hyg.* 59(2): 336-340.

Moffett, A., N. Shackelford, and S. Sarkar. 2007. Malaria in Africa: vector species' niche models and relative risk maps. *PLoS ONE*, 2:e824.

MoH. 2001. National Roll Back Malaria Strategic Plan (2001-2005). Ministry of Health. Lusaka, Zambia.

MoH. 2006. Ministry of Health: *Malaria Communication Strategy. Lusaka, Zambia*.

MoH. 2006. National Malaria Strategic Plan 2006-2011: A road map for RBM impact in Zambia. Ministry of Health.Lusaka.

MoH. 2006. Zambia National Malaria Indicator Survey Report, 2006, Ministry of Health, Lusaka, Zambia.

MoH. 2007. Zambia National Malaria Annual Report 2007. Ministry of Health, Lusaka. Zambia.

MoH. 2008. Guidelines on the Distribution and Utilization of Insecticide Treated Nets for Malaria Prevention and Control, Ministry of Health, Lusaka. Zambia.

MoH. 2008. Zambia National Malaria Indicator Survey Report, 2008, Ministry of Health, Lusaka. Zambia.

MoH. 2009. National Guidelines for Indoor Residual Spraying in Zambia, Ministry of Health, Luasaka. Zambia.

MoH. 2010. Indoor residual spraying (IRS) annual report, Ministry of Health, Lusaka. Zambia.

MoH . 2000. National Malaria Situation Analysis.Ministry of Health, Lusaka.Zambia. Lusaka.

Mohloi, P. M. 2006. Implementation of biochemical and molecular tools for resistance detection, monitoring and management of malaria vectors in Southern Africa. Ph.D Thesis. University of Liverpool. United Kingdom.

MoJ. 1944. Mosquito Extermination ACT, CAP 312. Ministry of Justice. Zambia.

MoJ. 1964. Mosquito Extermination ACT, CAP 537. Ministry of Justice. Zambia.

Molineaux, L. 1997. Malaria and mortality: some epidemiological considerations. *Ann. Trop Med Parasitol.* 91(7): 811-825.

Molineaux, L., D. A. Muir, H. C. Spencer, and W. H. Wernsdorfer. 1988. The epidemiology of malaria and its measurement In: Wernsdorfer WH, McGregor I, eds. Malaria: principles and practice of malariology. Edinburgh: Churchill Livingstone. 999-1089.

Moody, A. 2002. Rapid diagnostic tests for malaria parasites. *Clin. Microbiol Rev.* 15(1): 66-78.

Morgan, J. C., H. Irving, L. M. Okedi, A. Steven, and C. S. Wondji. 2010. Pyrethroid resistance in an Anopheles funestus population from Uganda. *PLoS. One.* 5(7): e11872.

Mouatcho, J. C., K. Hargreaves, L. L. Koekemoer, B. D. Brooke, S. V. Oliver, R. H. Hunt, and M. Coetzee. 2007. Indoor collections of the Anopheles funestus group (Diptera: Culicidae) in sprayed houses in northern KwaZulu-Natal, South Africa. *Malar. J.* 6:30.: 30.

Mouchet, J. 1988. Mini-review: agriculture and vector resistance. Insect Science and its Application. 9, 297-302.

Mouchet, J. 1998. [Origin of malaria epidemics on the plateaus of Madagascar and the mountains of east and south Africa]. *Bull. Soc. Pathol. Exot.* 91(1): 64-66.

Mouchet, J., O. Faye, J. Juivez, and S. Manguin. 1996. Drought and malaria retreat in the Sahel, west Africa. *Lancet.* 348(9043): 1735-1736.

Muirhead-Thomson, R. C. 1960. The significance of irritability, behaviouristic avoidance and allied phenomena in malaria eradication. Bull World Health Organ. 22, 721-734.

Mukabana, W. R., K. Kannady, G. M. Kiama, J. N. Ijumba, E. M. Mathenge, I. Kiche, G. Nkwengulila, L. Mboera, D. Mtasiwa, Y. Yamagata, S. van, I, B. G. Knols, S. W. Lindsay, C. M. Caldas de, H. Mshinda, M. Tanner, U. Fillinger, and G. F. Killeen**.** 2006**.** Ecologists can enable communities to implement malaria vector control in Africa. *Malar. J.* 5:9.: 9.

Murphy, S. C. and J. G. Breman**.** 2001**.** Gaps in the childhood malaria burden in Africa: cerebral malaria, neurological sequelae, anemia, respiratory distress, hypoglycemia, and complications of pregnancy. *Am J Trop Med Hyg.* 64(1-2 Suppl): 57-67.

N'guessan, R., V. Corbel, M. Akogbeto, and M. Rowland**.** 2007**.** Reduced efficacy of insecticide-treated nets and indoor residual spraying for malaria control in pyrethroid resistance area, Benin. *Emerg. Infect Dis.* 13(2): 199-206.

Naglaki, T. 1975. Conditions for the existence of clines. Genetics 80, 595-615.

Najera, J. A. 1999. Malaria control:Achievements, problems and Startegies.WHO/MAL/99.1087. World Health Organization.

Najera, J. A. 1989**.** Malaria and the work of WHO. *Bull. World Health Organ.* 67(3): 229-243.

Najera, J. A. 1990**.** Malaria control: present situation and need for historical research. *Parassitologia.* 32(2): 215-229.

Najera, J. A. 1999**.** Prevention and control of malaria epidemics. *Parassitologia.* 41(1-3): 339-347.

Najera, J. A. 2000**.** Epidemiology in the strategies for malaria control. *Parassitologia.* 42(1-2): 9-24.

Nauen, R. 2006. Insecticide resistance in public health pests; A challenge for effective vector control. Public Health Journal. 18, 8-15.

Nchinda, T. C. 1998**.** Malaria: a reemerging disease in Africa. *Emerg. Infect Dis.* 4(3): 398-403.

Ndo, C., C. Antonio-Nkondjio, A. Cohuet, D. Ayala, P. Kengne, I. Morlais, P. H. Awono-Ambene, D. Couret, P. Ngassam, D. Fontenille, and F. and Simard**.** 2010. Population genetic structure of the malaria vector *Anopheles nili* in sub-Saharan Africa. Malaria Journal 9, 161.

Neven, L. G. 2000. Physiological responses of insects to heat. Postharvest Biology and Technology. 21, 103-111.

Neville, C. G., E. S. Some, V. O. Mung'ala, W. Mutemi, I. New, K. Marsh, and et al. 1996. Insecticide-treated bednets reduce mortality and severe morbidity from malaria in children on the Kenyan coast. Tropical Medicine and International Health. 1, 139-146.

Newcomb, R. D., P. M. Campbell, D. L. Ollis, E. Cheah, R. J. Russell, and J. G. Oakeshott. A single amino acid substitution converts a carboxylesterase to an organophosphorus hydrolase and confers insecticide resistance on a blowfly. Proc Natl Acad Sci U S A. 94, 7464-7468. 1997.

Noor, A. M., A. A. Amin, W. S. Akhwale, and R. W. Snow. 2007. Increasing coverage and decreasing inequity in insecticide-treated bed net use among rural Kenyan children. *PLoS. Med.* 4(8): e255.

Noor, A. M., V. C. Kirui, S. J. Brooker, and R. W. Snow. 2009. The use of insecticide treated nets by age: implications for universal coverage in Africa. *BMC. Public Health.* 9:369.: 369.

Norris, D. E. 2002. Genetic markers for study of the anopheline vectors of human malaria. *Int. J. Parasitol.* 32(13): 1607-1615.

Nyarango, P. M., T. Gebremeskel, G. Mebrahtu, J. Mufunda, U. Abdulmumini, A. Ogbamariam, A. Kosia, A. Gebremichael, D. Gunawardena, Y. Ghebrat, and Y. Okbaldet. 2006. A steep decline of malaria morbidity and mortality trends in Eritrea between 2000 and 2004: the effect of combination of control methods. *Malar. J.* 5:33.: 33.

O'Meara, W. P., J. G. Breman, and F. E. McKenzie. 2005. The promise and potential challenges of intermittent preventive treatment for malaria in infants (IPTi). *Malar J.* 4:33.: 33.

O'Meara, W. P., W. E. Collins, and F. E. McKenzie. 2007. Parasite prevalence: a static measure of dynamic infections. *Am J Trop Med Hyg.* 77(2): 246-249.

O'Meara, W. P., T. W. Mwangi, T. N. Williams, F. E. McKenzie, R. W. Snow, and K. Marsh. 2008. Relationship between exposure, clinical malaria, and age in an area of changing transmission intensity. *Am J Trop Med Hyg.* 79(2): 185-191.

Oakeshott, J. G., E. A. van Papenrecht, T. M. Boyce, M. J. Healy, and R. J. Russell. 1993. Evolutionary genetics of Drosophila esterases. Genetica. 90, 239-268.

Okara, R. M., M. E. Sinka, N. Minakawa, C. M. Mbogo, S. I. Hay, and R. W. Snow. 2010. Distribution of the main malaria vectors in Kenya. *Malar. J.* 9:69.: 69.

Okech, B. A., I. K. Mwobobia, A. Kamau, S. Muiruri, N. Mutiso, J. Nyambura, C. Mwatele, T. Amano, and C. S. Mwandawiro. 2008. Use of integrated malaria management reduces malaria in Kenya. *PLoS. One.* 3(12): e4050.

Okiro, E. A., S. I. Hay, P. W. Gikandi, S. K. Sharif, A. M. Noor, N. Peshu, K. Marsh, and R. W. Snow. 2007. The decline in paediatric malaria admissions on the coast of Kenya. *Malar J.* 6:151.: 151.

Omumbo, J., J. Ouma, B. Rapuoda, M. H. Craig, S. D. le, and R. W. Snow. 1998. Mapping malaria transmission intensity using geographical information systems (GIS): an example from Kenya. *Ann Trop Med Parasitol.* 92(1): 7-21.

Oppenoorth, F. J. 1985. Biochemical and genetic in insecticide resistance. In:

Comprehensive Insect Physiology Biochemistry and Pharmacology (eds. Kerkut, G.A. and Gilbert L.I.) Pergamon Press. pp 731-773.

Pages, F., E. Orlandi-Pradines, and V. Corbel**.** 2007**.** [Vectors of malaria: biology, diversity, prevention, and individual protection]. *Med Mal Infect.* 37(3): 153-161.

Pampana, E. J. 1969. A textbook of malaria eradication. Oxford University Press, London.

Pardo, G., M. A. Descalzo, L. Molina, E. Custodio, M. Lwanga, C. Mangue, J. Obono, A. Nchama, J. Roche, A. Benito, and J. Cano**.** 2006**.** Impact of different strategies to control Plasmodium infection and anaemia on the island of Bioko (Equatorial Guinea). *Malar. J.* 5:10.: 10.

Paterson, H. E. 1963. The species, species control and antimalarial spraying campaigns, implications of recent work on the *An. gambiae* complex**.** South African Journal of Medical Science. 28, 33-44.

Pates, H. and C. Curtis**.** 2005. Mosquito behavior and vector control. Annu Rev Entomol. 50, 53-70.

Paul, D. 1992. Fitness: historical perspective. *In:* Keller, E.F. and Lloyd, E.A. eds. *Keywords in evolutionary biology*. Harvard University Press, Cambridge, 112-114.

Penilla, R. P., A. D. Rodriguez, J. Hemingway, J. L. Torres, J. I. Arredondo-Jimenez, and M. H. Rodriguez**.** 1998**.** Resistance management strategies in malaria vector mosquito control. Baseline data for a large-scale field trial against Anopheles albimanus in Mexico. *Med Vet Entomol.* 12(3): 217-233.

Pielou, D. P. 1947. Anopheline mosquitoes breeding in fish dams, pools, and streams in Northern Rhodesia. Proc.R.Entomol.Soc.London. 22, 18-23.

Pimentel, D. and A. C. Bellotti**.** 1976. Parasite-host population systems and geneti stability. Am.Nat. 110, 877-888.

Pinto, J., M. J. Donnelly, C. A. Sousa, V. Gil, C. Ferreira, N. Elissa, V. E. do Rosario, and J. D. Charlwood**.** 2002**.** Genetic structure of Anopheles gambiae (Diptera: Culicidae) in Sao Tome and Principe (West Africa): implications for malaria control. *Mol. Ecol.* 11(10): 2183-2187.

Pluess, B., F. C. Tanser, C. Lengeler, and B. L. Sharp**.** 2010**.** Indoor residual spraying for preventing malaria. *Cochrane. Database. Syst. Rev.* 4:CD006657.: CD006657.

Povoa, M. M., R. L. D. Machado, M. N. O. Segura, G. M. R. Vianna, A. S. Vasconcelos, and Conn.J.E. 2000. Infectivity of malaria vector mosquitoes: correlation of positivity between ELISA and PCR-ELISA tests. Trans.R.Soc.Trop.Med.Hyg. 94, 106-107.

Prapanthadara, L., J. Hemingway, and A. J. Ketterman**.** 1993. Partial purification and characterization of glutathione S-transferase involved in DDT resistance from the mosquito *Anopheles gambiae*. Pest.Biochem.Physiol. 47, 119-133.

Protopopoff, N., B. W. Van, T. Marcotty, H. M. Van, P. Maes, D. Baza, U. D'Alessandro, and M. Coosemans. 2007. Spatial targeted vector control in the highlands of Burundi and its impact on malaria transmission. *Malar J.* 6:158.: 158.

Protopopoff, N., B. W. Van, T. Marcotty, H. M. Van, P. Maes, D. Baza, U. D'Alessandro, and M. Coosemans. 2008. Spatial targeted vector control is able to reduce malaria prevalence in the highlands of Burundi. *Am J Trop Med Hyg.* 79(1): 12-18.

Pull, J. H. 1972. Malaria surveillance methods, their uses and limitations. *Am J Trop Med Hyg.* 21(5): 651-657.

Ranson, H., H. Abdallah, A. Badolo, W. M. Guelbeogo, C. Kerah-Hinzoumbe, E. Yangalbe-Kalnone, N. Sagnon, F. Simard, and M. Coetzee. 2009. Insecticide resistance in Anopheles gambiae: data from the first year of a multi-country study highlight the extent of the problem. *Malar. J.* 8(1): 299.

Ranson, H., C. Claudianos, F. Ortelli, C. Abgrall, J. Hemingway, M. V. Sharakhova, M. F. Unger, F. H. Collins, and R. Feyereisen. 2002. Evolution of supergene families associated with insecticide resistance. *Science.* 298(5591): 179-181.

Ranson, H., Vulule J.M., X. ang, J. Hemingway, and a. C. F. H. 2000. Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan Anophelse gambiae associated with resistance to DDT and pyrethroids. Insect Molecular Biology 9, 491-497.

Rao, J. N. K. and A. J. Scott. 1981. The analysis of categorical data from complex sample surveys: chi-squared tests for goodness of fit and independence in two-way tables. J Am Stat Assoc 76, 221-230.

Rawlings, P., G. Davidson, R. K. Sakai, H. R. Rathor, M. Aslamkhan, and C. F. Curtis. 1981. Field measurement of the effective dominance of an insecticide resistance in anopheline mosquitos. *Bull World Health Organ.* 59(4): 631-640.

Ray, D. E. 1991. Pesticides derived from plants and other organisms. In W.J. Hayes, Jr and E.R. Laws (Eds), Handbook of Pesticide Toxicology. 2.

Raymond, M., C. Chevillon, T. Guillemaud, Lenormand T, and N. Pasteur. 1998. An overview of the evolution of overproduced esterases in the mosquito *Culex pipiens*. *Philos.* Trans.R.Soc.London 353, 1707-1711.

RBM. 2000. *Framework for monitoring progress and evaluating outcomes and impact*. Geneva, World Health Organization.

Reyburn, H., R. Mbatia, C. Drakeley, J. Bruce, I. Carneiro, R. Olomi, J. Cox, W. M. Nkya, M. Lemnge, B. M. Greenwood, and E. M. Riley. 2005. Association of transmission intensity and age with clinical manifestations and case fatality of severe Plasmodium falciparum malaria. *JAMA.* 293(12): 1461-1470.

Ribeiro, J. M. C., F. Seulu, T. Abose, G. Kidane, and A. Teklehaimanot. 1996. Temporal and spatial distribution of anopheline mosquitoes in an Ethiopian village: implications for malaria control strategies. Bull World Health Organ. 74, 299-305.

Ridley, R. G. 2002. Introduction. Antimalarial drug resistance: ramifications, explanations and challenges. *Microbes. Infect.* 4(2): 155-156.

Rihet, P., Y. Traore, L. Abel, C. Aucan, T. Traore-Leroux, and F. Fumoux. 1998. Malaria in humans: Plasmodium falciparum blood infection levels are linked to chromosome 5q31-q33. *Am J Hum. Genet.* 63(2): 498-505.

Rishikesh, N. 1966. Observations on Anopheline of Malaria in an Upland Valley in Ethiopia.(Unpublished document, WHO/MAL/66554) WHO, Geneva.

Rivola, E. and M. H. Holstein. Note sur une variete d'*Anopheles nili* Theo. Bull Soc Pathol Exot. 50, 382-387. 1957.

Robert, V. and P. Carnevale. 1991. Influence of deltamethrin treatment of bed nets on malaria transmission in the Kou valley, Burkina Faso. Bull World Health Organ. 69, 735-740.

Robert, V., K. Macintyre, J. Keating, J. F. Trape, J. B. Duchemin, M. Warren, and J. C. Beier. 2003. Malaria transmission in urban sub-Saharan Africa. *Am. J. Trop. Med. Hyg.* 68(2): 169-176.

Roberts, D., C. Curtis, R. Tren, B. Sharp, C. Shiff, and R. Bate. 2004. Malaria control and public health. *Emerg. Infect Dis.* 10(6): 1170-1171.

Roberts, D. R. and R. G. Andre. 1994. Insecticide resistance issues in vector-borne disease control. *Am. J. Trop. Med. Hyg.* 50(6 Suppl): 21-34.

Roberts, J. M. 1964. The control of epidemic malaria in the highlands of western Kenya. 3. After the campaign. *J Trop Med Hyg.* 67:230-7.: 230-237.

Rodrigues, F. G., M. N. Santos, T. X. de Carvalho, B. C. Rocha, M. A. Riehle, P. F. Pimenta, E. G. Abraham, M. Jacobs-Lorena, C. F. Alves de Brito, and L. A. Moreira. 2008. Expression of a mutated phospholipase A2 in transgenic Aedes fluviatilis mosquitoes impacts Plasmodium gallinaceum development. *Insect Mol. Biol.* 17(2): 175-183.

Rodriguez, A. D., R. P. Penilla, M. H. Rodriguez, and Hemingway J. 2006. Insecticide resistance management in a multi-resistant malaria vector scenario. A Mexican trial shows sustainability. Public Health Journal. 18, 24-29.

Rogers, D. J., S. E. Randolph, R. W. Snow, and S. I. Hay. 2002. Satellite imagery in the study and forecast of malaria. *Nature.* 415(6872): 710-715.

Rogier, C., M. C. Henry, and J. F. Trape. 2009. [Epidemiologic evaluation of malaria in endemic areas]. *Med Trop (Mars.).* 69(2): 123-142.

Ross, R. The Prevention of Malaria. London: Murray. 1911.

Roush, R. T. and J. A. Mckenzie. 1987. Ecological genetics of insectiicide and acaricide resistance. Ann Rev of Entomol. 32, 361-380.

Rowland, M., S. Hewitt, N. Durrani, P. Saleh, M. Bouma, and E. Sondorp. 1997.

Sustainability of pyrethroid-impregnated bednets for malaria control in Afghan communities. *Bull World Health Organ.* 75(1): 23-29.

Rozendaal, J. A. Vector control. 1997. Methods for the use by Individuals and communities. Geneva: World Health Organization.

Russell, R. J., G. C. Robin, P. Kostakos, R. D. Newcomb, T. M. Boyce, and K. M. e. al. Medveczky**.** 1996. Molecular cloning of an a-esterase gene cluster on chromosome 3R of Drosophila melanogaster. Insect Biochem Mol Biol. 26, 235-247.

Sachs, J. and P. Malaney**.** 2002**.** The economic and social burden of malaria. *Nature.* 415(6872): 680-685.

Sampath, T. R., R. S. Yadav, V. P. Sharma, and T. Adak**.** 1998**.** Evaluation of lambdacyhalothrin-impregnated bednets in a malaria endemic area of India. Part 2. Impact on malaria vectors. *J Am Mosq Control Assoc.* 14(4): 437-443.

Saul, A. 1993**.** Minimal efficacy requirements for malarial vaccines to significantly lower transmission in epidemic or seasonal malaria. *Acta Trop.* 52(4): 283-296.

Scott, J. A., W. G. Brogdon, and F. H. Collins**.** 1993**.** Identification of single specimens of the Anopheles gambiae complex by the polymerase chain reaction. *Am. J. Trop. Med. Hyg.* 49(4): 520-529.

Scott, J. G., D. G. Cochran, and B. D. Siefried**.** 1990. Insecticide toxicity, synergism, and resistance in the Germany cockroach, Blattella germanica (Dictyoptera: Blattellidae). Journal of Economic Entomology 83, 1698-1703.

Service, M. W. 1971. Studies on sampling larval populations of the *Anopheles gambiae* complex. Bull World Health Organ. 45, 169-180.

Service, M. W. 1977. A critical review of procedures for sampling populations of adult mosquitoes. Bull.Entomol.Res. 67, 343-382.

Service, M. W. 2000. Medical entomology for students. Liverpool: Cambridge University Press.

Service, M. W. 1976. Mosquito ecology: Field sampling methods. Applied Science Publishers Ltd. London.

Sharp, B., W. P. van, J. B. Sikasote, P. Banda, and I. Kleinschmidt**.** 2002**.** Malaria control by residual insecticide spraying in Chingola and Chililabombwe, Copperbelt Province, Zambia. *Trop. Med. Int. Health.* 7(9): 732-736.

Sharp, B. L. and D. Le Sueur**.** 1996**.** [Malaria in South Africa: past, present and perspectives]. *Med. Trop. (Mars.).* 56(2): 189-196.

Sharp, B. L., F. C. Ridl, D. Govender, J. Kuklinski, and I. Kleinschmidt**.** 2007**.** Malaria vector control by indoor residual insecticide spraying on the tropical island of Bioko, Equatorial Guinea. *Malar. J.* 6:52.: 52.

Shaukat, A. M., J. G. Breman, and F. E. McKenzie**.** 2010**.** Using the entomological inoculation rate to assess the impact of vector control on malaria parasite transmission and elimination. *Malar. J.* 9:122.: 122.

Shelly, A. J. 1973. Observations on the behaviour of *Anopheles gambiae* species B in Kambole Village in the Zambezi Valley, Zambia. Annals of Tropical Medicine Parasitology. 67, 237-248.

Shiff, C., W. Checkley, P. Winch, Z. Premji, J. Minjas, and P. Lubega**.** 1996**.** Changes in weight gain and anaemia attributable to malaria in Tanzanian children living under holoendemic conditions. *Trans. R Soc Trop Med Hyg.* 90(3): 262-265.

Shiff, C. J., J. N. Minjas, T. Hall, R. H. Hunt, S. Lyimo, and et al. 1995. Malaria infection potential of anopheline mosquitoes sampled by light trapping indoors in coastal Tanzanian villages. 9. Med Vet Entomol., 256-262.

Shililu, J., T. Ghebremeskel, F. Seulu, S. Mengistu, H. Fekadu, M. Zerom, G. E. Asmelash, D. Sintasath, C. Mbogo, J. Githure, E. Brantly, J. C. Beier, and R. J. Novak**.** 2004**.** Seasonal abundance, vector behavior, and malaria parasite transmission in Eritrea. *J. Am. Mosq. Control Assoc.* 20(2): 155-164.

Shousha, A. T. 1948. Species-eradication. the eradication of Anopheles gambiae from Upper Egypt, 1942-1945. Bull World Health Organ. 1, 309-353.

Siachinji, V. and M. Mulenga**.** 2002. Entomological studies in Southern province of Zambia to determine major vectors of malaria. Abstract of the 23rd Annual Scientific and General meeting of the Entomological Society of Zambia. 30th January 2004. Andrews Motel, Lusaka, Zambia.

Siachinji, V., L. Mwanza, A. Enerst, and N. Mataa**.** 2001. Report of the Evaluation of Malaria Vector Susceptibility to Insecticides in Zambia. Tropical Disease Research Centre. Zambia.

Silver, J. B. 2008. Mosquito ecology: Field Sampling Methods. Springer Science.

Singh, G. P., S. Chitkara, N. L. Kalra, K. B. Makepur, and M. V. Narasimham**.** 1990**.** Development of a methodology for malariogenic stratification as a tool for malaria control. *J Commun. Dis.* 22(1): 1-11.

Sipilanyambe, N., J. L. Simon, P. Chanda, P. Olumese, R. W. Snow, and D. H. Hamer**.** 2008**.** From chloroquine to artemether-lumefantrine: the process of drug policy change in Zambia. *Malar. J.* 7:25.: 25.

Smith, D. L., J. Dushoff, R. W. Snow, and S. I. Hay**.** 2005**.** The entomological inoculation rate and Plasmodium falciparum infection in African children. *Nature.* 438(7067): 492-495.

Smith, D. L., C. A. Guerra, R. W. Snow, and S. I. Hay**.** 2007**.** Standardizing estimates of the Plasmodium falciparum parasite rate. *Malar J.* 6:131.: 131.

Smith, D. L., S. I. Hay, A. M. Noor, and R. W. Snow**.** 2009**.** Predicting changing malaria risk after expanded insecticide-treated net coverage in Africa. *Trends*

Parasitol. 25(11): 511-516.

Smith, D. L. and F. E. McKenzie**.** 2004**.** Statics and dynamics of malaria infection in Anopheles mosquitoes. *Malar. J.* 3:13.: 13.

Smith, T., J. D. Charlwood, J. Kihonda, S. Mwankusye, P. Billingsley, J. Meuwissen, E. Lyimo, W. Takken, T. Teuscher, and M. Tanner**.** 1993**.** Absence of seasonal variation in malaria parasitaemia in an area of intense seasonal transmission. *Acta Trop.* 54(1): 55-72.

Smith, T., J. D. Charlwood, W. Takken, M. Tanner, and D. J. Spiegelhalter**.** 1995**.** Mapping the densities of malaria vectors within a single village. *Acta Trop.* 59(1): 1-18.

Smith, T., B. Genton, K. Baea, N. Gibson, J. Taime, A. Narara, F. Al-Yaman, H. P. Beck, J. Hii, and M. Alpers**.** 1994**.** Relationships between Plasmodium falciparum infection and morbidity in a highly endemic area. *Parasitology.* 109(Pt 5): 539-549.

Smith, T. A., Leuenberger R, and Lengeler C**.** 2001. Child mortality and malaria transmission intensity in Africa. Trends Parasitol. 17, 145-149.

Snow, R. W., M. Craig, Deichmann U, and Marsh K**.** 1999. Estimating mortality, morbidity and disability due to malaria among Africa's non-pregnant population. Bull World Health Organ. 77, 617-618.

Snow, R. W., C. A. Guerra, J. J. Mutheu, and S. I. Hay**.** 2008**.** International funding for malaria control in relation to populations at risk of stable Plasmodium falciparum transmission. *PLoS. Med.* 5(7): e142.

Snow, R. W., C. A. Guerra, A. M. Noor, H. Y. Myint, and S. I. Hay**.** 2005. The global distribution of clinical episodes of Plasmodium falciparum malaria. Nature (Lond.). 434, 214-217.

Snow, R. W. and K. Marsh**.** 2002**.** The consequences of reducing transmission of Plasmodium falciparum in Africa. *Adv. Parasitol.* 52:235-64.: 235-264.

Snow, R. W., J. A. Omumbo, B. Lowe, C. S. Molyneux, J. O. Obiero, A. Palmer, M. W. Weber, M. Pinder, B. Nahlen, C. Obonyo, C. Newbold, S. Gupta, and K. Marsh**.** 1997**.** Relation between severe malaria morbidity in children and level of Plasmodium falciparum transmission in Africa. *Lancet.* 349(9066): 1650-1654.

Soderlund, D. M. and J. R. Bloomquist**.** 1989. 1989. Neurotoxic action of pyrethroid insecticides. Annu.Rev.Entomol. 34, 77-96.

Soderlung, D. M., J. R. Bloomquist, R. T. Roush, and B. E. Tabashnik**.** 1990. Molecular mechanisms of insecticide resistance. In: Pesticide Resistance in Arthropods. Chapman and Hall, New York and London.

Somboon, P., N. Morakote, S. Koottathep, and Trisanarom.U. 1993. Detection of sporozoites of *Plasmodium vivax* and *Plasmodium falciparum* in mosquitoes by ELISA: false positivity associated with bovine and swine blood. Trans.R.Soc.Trop.Med.Hyg. 87, 322-324.

Some, E. S., D. K. Koech, J. O. Ochogo, F. Ocholla, and F. Mumbi**.** 1997**.** An evaluation of surveillance of malaria at primary health care level in Kenya. *East Afr. Med J.* 74(9): 573-575.

Soper, F. L. and D. B. Wilson**.** 1943. *Anopheles gambiae* in Brazil.New York: The Rockefeller Foundation.

Sparks, T. C., J. A. Lockwood, R. L. Byford, J. B. Graves, and B. R. Leonard**.** 1989. The role of behaviour in insecticide resistance. Pestic.Sci. 26, 383-399.

Spillings, B. L., B. D. Brooke, L. L. Koekemoer, J. Chiphwanya, M. Coetzee, and R. H. Hunt**.** 2009**.** A new species concealed by Anopheles funestus Giles, a major malaria vector in Africa. *Am J Trop Med Hyg.* 81(3): 510-515.

Stewart, L., R. Gosling, J. Griffin, S. Gesase, J. Campo, R. Hashim, P. Masika, J. Mosha, T. Bousema, S. Shekalaghe, J. Cook, P. Corran, A. Ghani, E. M. Riley, and C. Drakeley**.** 2009**.** Rapid assessment of malaria transmission using age-specific sero-conversion rates. *PLoS. One.* 4(6): e6083.

Storrs, A. G. Know your trees. 1995. Regional Conservation Unit. Nairobi, Kenya.

Struik, S. S. and E. M. Riley**.** 2004**.** Does malaria suffer from lack of memory? *Immunol. Rev.* 201:268-90.: 268-290.

Surtees, G. 1970. Large-scale irrigation and Arbovirus epidemiology, Kano Plain, Kenya. I.Description of the area and preliminary studies on the mosquitoes. J Med Entomol. 7, 509-517.

Sutherland, C. J., R. Ord, S. Dunyo, M. Jawara, C. J. Drakeley, N. Alexander, R. Coleman, M. Pinder, G. Walraven, and G. A. Targett**.** 2005**.** Reduction of malaria transmission to *Anopheles* mosquitoes with a six-dose regimen of co-artemether. *PLoS. Med.* 2(4): e92.

Sutherst, R. W. and G. F. Maywald**.** 1995. Predicting insect distributions in a changed climate. *In:* Harrington, R. and Stork, N. E. eds. *Insects in a changing environment : 17th symposium of the Royal Entomological Society of London, 7-10 September 1993 at Rothamsted Experimental Station, Harpenden*. Academic Press, London, 59-61.

Sweeney, A. W. 1996**.** The possibility of an "X" factor. The first documented drug resistance of human malaria. *Int. J. Parasitol.* 26(10): 1035-1061.

Tabashnik, B. E. 1989. Managing resistance with multiple pesticide tactics: theory, evidence and recommendations. Journal of Economic Entomology. 82, 1263-1269.

Takken, W. 2002**.** Do insecticide-treated bednets have an effect on malaria vectors? *Trop. Med. Int. Health.* 7(12): 1022-1030.

Takken, W., M. J. Klowden, and G. M. Chambers**.** 1998**.** Effect of body size on host seeking and blood meal utilization in Anopheles gambiae sensu stricto (Diptera: Culicidae): the disadvantage of being small. *J. Med. Entomol.* 35(5): 639-645.

Taylor, C. E., F. Quaglia, and G. P. Georghiou. 1983. Evolution of resistance to insecticides: a cage study on the influence of migration and insecticide decay rates. Journal of Economic Entomology. 76, 704-706.

Taylor, P. and S. L. Mutambu. 1986. A review of the malaria situation in Zimbabwe with special reference to the period 1972-1981. *Trans R Soc Trop Med Hyg.* 80(1): 12-19.

Teklehaimanot, A., A. Kassahun, and M. Fletcher. 1993. Using fish against malaria: a local initiative. *World Health Forum.* 14(2): 176-177.

Thomas, C. J. and S. W. Lindsay. 2000. Local-scale variation in malaria infection amongst rural Gambian children estimated by satellite remote sensing. Trans R Soc Trop Med Hyg. 94, 159-163.

Tomita, T. and J. G. Scott. 1995. cDNA and deduced protein sequence of Cyp6D1: the putative gene for a cytochrome P450 responsible for pyrethroid resistance in house fly. Insect Biochem Mol Biol. 25, 275-283.

Toure, Y. T., G. Dolo, V. Petrarca, S. F. Traore, M. Bouare, A. Dao, J. Carnahan, and C. E. Taylor. 1998. Mark-release-recapture experiments with Anopheles gambiae s.l. in Banambani Village, Mali, to determine population size and structure. *Med Vet. Entomol.* 12(1): 74-83.

Toure, Y. T., V. Petrarca, S. F. Traore, A. Coulibaly, H. M. Maiga, O. Sankare, M. Sow, M. A. Di Deco, and M. Coluzzi. 1994. Ecological genetic studies in the chromosomal form Mopti of Anopheles gambiae s.str. in Mali, west Africa. *Genetica.* 94(2-3): 213-223.

Townson, H., M. B. Nathan, M. Zaim, P. Guillet, L. Manga, R. Bos, and M. Kindhauser. 2005. Exploiting the potential of vector control for disease prevention. *Bull. World Health Organ.* 83(12): 942-947.

Trape, J. F. and C. Rogier. 1996. Combating malaria morbidity and mortality by reducing transmission. *Parasitol Today.* 12(6): 236-240.

Trigg, P. I. and A. V. Kondrachine. 1998. Commentary: malaria control in the 1990s. *Bull. World Health Organ.* 76(1): 11-16.

Utzinger, J., Y. Tozan, F. Doumani, and B. H. Singer. 2002. The economic payoffs of integrated malaria control in the Zambian copperbelt between 1930 and 1950. *Trop. Med. Int. Health.* 7(8): 657-677.

Utzinger, J., Y. Tozan, and B. H. Singer. 2001. Efficacy and cost-effectiveness of environmental management for malaria control. *Trop. Med. Int. Health.* 6(9): 677-687.

Van den Berg, H. and W. Takken. 2007. A framework for decision-making in integrated vector management to prevent disease. Trop.Med.Int.Health. 12, 1230-1238.

Vaughan, A., N. Hawkes, and J. Hemingway. 1997. Co-amplification explains

linkage disequilibrium of two mosquito esterase genes in insecticide-resistant *Culex quinquefasciatus*. Biochem.J. 325, 359-365.

Vaughan, A. and J. Hemingway**.** 1995. Mosquito carboxylesterase Esta21 (A2). Cloning and sequence of the full length cDNA for a major insecticide resistance gene worldwide in the mosquito *Culex quinquefasciatus*. J.Biol.Chem. 270, 17044-17049.

Vetter, W. and G. Scherer**.** 1999. Persistency of toxaphene components in mammals that can be explained by molecular modelling. Environ.Sci.Technol. 33, 3458-3461.

Vulule, J. M., R. F. Beach, F. K. Atieli, J. M. Roberts, D. L. Mount, and R. W. Mwangi**.** 1994**.** Reduced susceptibility of Anopheles gambiae to permethrin associated with the use of permethrin-impregnated bednets and curtains in Kenya. *Med Vet Entomol.* 8(1): 71-75.

Walker, K. 2000**.** Cost-comparison of DDT and alternative insecticides for malaria control. *Med Vet Entomol.* 14(4): 345-354.

Wang-Sattler, R., S. Blandin, Y. Ning, C. Blass, G. Dolo, Y. T. Toure, T. A. delle, G. C. Lanzaro, L. M. Steinmetz, F. C. Kafatos, and L. Zheng**.** 2007**.** Mosaic genome architecture of the Anopheles gambiae species complex. *PLoS. One.* 2(11): e1249.

Ware, G. W. 2010. Pesticides. Theory and application. W.H. Freeman and Company. San Fransisco California.

Warrell, D. A. and H. M. Gillies**.** Essential malariology. Fourth edition. New York: Arnold. 2002.

Watson, M. African Highway: The Battle for Health in Central Africa. John Murray, London. 1953.

Weeto, M. M., L. L. Koekemoer, L. Kamau, R. H. Hunt, and M. Coetzee**.** 2004. Evaluation of a species-specific PCR assay for the *Anopheles funestus* group from eleven African countries and Madagascar. Trans.Royal Soc.Trop.Med.Hyg. 98, 142-147.

White, G. B. and P. Rosen**.** 1973. Comparative studies on sibling species of the *Anopheles gambiae* Giles complex (Dipt., Culicidae). II. Ecology of species A and B in savanna around Kaduna, Nigeria, during transition from wet to dry season. *Bulletin of Entomological Research* **62**:613-625.

White, G. B. 1974**.** Anopheles gambiae complex and disease transmission in Africa. *Trans. R. Soc. Trop. Med. Hyg.* 68(4): 278-301.

White, N. J. 1998**.** Preventing antimalarial drug resistance through combinations. *Drug Resist. Updat.* 1(1): 3-9.

W.H.O**.** 1975. Manual on practical entomology in malaria. PartII: Methods and Techniques.

W.H.O**.** 1998. Test Procedures for Insecticide Resistance Monitoring in Malaria

Vectors, Bio-efficacy and Persistence of insecticides in treated surfaces. Report of the WHO Informal Consultation. WHO/CDS/CPC/MAL/98.12. World Health Organization, Geneva, Switzerland.

W.H.O. 2000. Techniques to detect insecticide resistance mechanisms (field and laboratory manual), WHO, Geneva.

W.H.O. 2003. Minutes MERG Anaemia Task Force Meeting: 27-28th Oct 2003. Geneva: World Health Organization/Roll Back Malaria.

WHO. 1957. Insecticides. 7th report of the expert committee on insecticides. WHO Technical Report Series 125.

WHO. 1963. Criteria and meaning of tests for determining susceptibility or resistance of insects to insecticides. WHO Technical Report series 265, 135-138.

WHO. 1963. Terminology of malaria and of malaria eradication. Report of a drafting committee. Geneva: World Health Organization, 1963.

WHO. 1980. Resistance of vectors of disease to pesticides. 5th report of expert committee on vector biology and control. WHO Technical Report series 655.

WHO. 1993. World Health Organization: *A global strategy for malaria control*. Geneva.

WHO. 1995. Supplies for monitoring insecticide resistance in disease vectors. Geneva, Switzerland.

WHO. 2001. Chemistry and specifications of pesticides.World Health Organization. Tech Rep Ser 2001 899, 1-68.

WHO. 2001. WHO-Action plan for the reduction of reliance on DDT in Disease Vector Control. Geneva: World Health Organization;

WHO. 2001. World Health Organization Commission on Macroeconomics and Health: Macroeconomics and Health: investing in health for economic development. In *Report of the Commission on Macroeconomics and Health* World Health Organization. Geneva.

WHO. 2003. Africa Malaria Report 2003. Geneva: World Health Organization,Document WHO/CDS/MAL/2003.1093.

WHO. 2003. Guidelines for integrated vector management. Harare, World Health Organization Regional Office for Africa.

WHO. 2004. Global Strategic Framework for Integrated Vector Management. Geneva: World Health Organization.

WHO. 2005. World malaria report 2005. WHO/HTM/MAL/2005.1102.

WHO. 2006. *Indoor residual spraying-Use of indoor residual spraying for scaling up global malaria control and elimination.* Geneva, World Health Organization.

WHO. 2006. Informal consultation on malaria elimination: setting up the WHO agenda. WHO/HTM/MAL/2006.1114. Geneva: World Health Organization.

WHO. 2007. *Global malaria indicators and their measurement*. Geneva, World Health Organization.

WHO. 2007. Insecticide-treated mosquito nets: a WHO position statement. Geneva, World Health Organization, Global Malaria Programme.

WHO. 2007. Malaria elimination: a field manual for low and moderate endemic countries. Geneva: World Health Organization.

WHO. 2008. *Targets for malaria control*. Geneva, World Health Organization.

WHO. 2008. World malaria report 2008. WHO/HTM/GMP/2008.1.

WHO. 2009. World Health Organization News Release: Malaria deaths plunge by 66% in Zambia. 23 April 2009/Geneva/Brazzaville.

WHO. 2009. *World malaria report 2009.* Geneva, World Health Organization.

WHO. 1971. Technical guide for a system of malaria surveillance (application of resolution WHA22.47). Wkly Epidemiol Rec. 72, 329-333.

WHO. 2005. Atlas of insecticide resistance in malaria vectors of WHO African region. African Network for vector resistance (ANVR). Harare, Zimbabwe.

WHOPES a. 2007. WHO Pesticides Evaluation Scheme (WHOPES). WHO recommended insecticides for indoor residual spraying against malaria vectors. www.who.int/malaria/cmcupload/0/000/012/ 604/IRSInsecticides.htm.

WHOPES b. 2007. WHO Pesticide Evaluation Scheme (WHOPES). WHO recommended insecticide products treatment of mosquito nets for malaria vector. [http://www.who.int/whopes/en/].

Wilkes, T. J., Y. G. Matola, and J. D. Charlwood. 1996. Anopheles rivulorum, a vector of human malaria in Africa. *Med. Vet. Entomol.* 10(1): 108-110.

Wilkinson, C. F. 1976. Insecticide interactions. Wilkinson CF, ed.*Insecticide Biochemistry and Physiology*. New York: Plenum Press, 605-647.

Williamson, M. S., D. Martinez-Torres, C. A. Hick, and A. L. Devonshire. 1996. Identification of mutations in the housefly para-type sodium channel gene associated with knockdown resistance (kdr) to pyrethroid insecticides. Mol.Gen.Genet. 245, 51-60.

Wirtz, R. A., Y. Charoenvit, T. R. Burkot, and e. al. 1987. Comparative testing of *Plasmodium falciparum* sporozoite monoclonal antibodies for ELISA development. Bulletin of the World Health Organization. 65, 39-45.

Wondji, C., F. Simard, and ontenille D. 2002. Evidence for genetic differentiaon between the molecular forms M and S within the Forest chromosomal form of

Anopheles gambiae in an area of sympatry. Insect Molecular Biology. 11, 11-19.

Wondji, C. S., H. Irving, J. Morgan, N. F. Lobo, F. H. Collins, R. H. Hunt, M. Coetzee, J. Hemingway, and H. Ranson**.** 2009**.** Two duplicated P450 genes are associated with pyrethroid resistance in Anopheles funestus, a major malaria vector. *Genome Res.* 19(3): 452-459.

Yadav, R. S., T. R. Sampath, V. P. Sharma, T. Adak, and S. K. Ghosh**.** 1998**.** Evaluation of lambdacyhalothrin-impregnated bednets in a malaria endemic area of India. Part 3. Effects on malaria incidence and clinical measures. *J Am Mosq Control Assoc.* 14(4): 444-450.

Yadouleton, A. W., A. Asidi, R. F. Djouaka, J. Braima, C. D. Agossou, and M. C. Akogbeto**.** 2009**.** Development of vegetable farming: a cause of the emergence of insecticide resistance in populations of Anopheles gambiae in urban areas of Benin. *Malar. J.* 8:103.: 103.

Yebakima, A., M. Marquine, J. Rosine, M. M. Yp-Tcha, and N. Pasteur**.** 2004**.** Evolution of resistance under insecticide selection pressure in Culex pipiens quinquefasciatus (Diptera, Culicidae) from Martinique. *J Med Entomol.* 41(4): 718-725.

Yekutiel, P. 1960. Problems of epidemiology in malaria eradication. Bull World Health Organ. 22, 669-683.

Yohannes, M., M. Haile, T. A. Ghebreyesus, K. H. Witten, A. Getachew, P. Byass, and S. W. Lindsay**.** 2005**.** Can source reduction of mosquito larval habitat reduce malaria transmission in Tigray, Ethiopia? *Trop. Med. Int. Health.* 10(12): 1274-1285.

Yukich, J. O., C. Lengeler, F. Tediosi, N. Brown, J. A. Mulligan, D. Chavasse, W. Stevens, J. Justino, L. Conteh, R. Maharaj, M. Erskine, D. H. Mueller, V. Wiseman, T. Ghebremeskel, M. Zerom, C. Goodman, D. McGuire, J. M. Urrutia, F. Sakho, K. Hanson, and B. Sharp**.** 2008**.** Costs and consequences of large-scale vector control for malaria. *Malar J.* 7:258.: 258.

Zahar, A. R. 1985. Vector bionomics in the epidemiology and control of malaria. Part I, The WHO African Region & the Southern WHO eastern Mediterranean Region. Section III. (Equatorial Africa) (Southern Africa). WHO/VBC/85.2. World Health Organization, Geneva.

Zlotkin, E. 1999. The insect voltage-gated sodium channel as target of insecticides. Annual Review of Entomology. 44, 429-455.

Zurovac, D., B. Midia, S. A. Ochola, M. English, and R. W. Snow**.** 2006**.** Microscopy and outpatient malaria case management among older children and adults in Kenya. *Trop Med Int Health.* 11(4): 432-440.